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# Role of Physical Heterogeneity in the Interpretation of Small-Scale Laboratory and Field Observations of Bacteria, Microbial-Sized Microsphere, and Bromide Transport Through Aquifer Sediments

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The effect of physical variability upon the relative transport behavior of microbial-sized microspheres, indigenous bacteria, and bromide was examined in field and flow-through column studies for a layered, but relatively well sorted, sandy glaciofluvial aquifer. These investigations involved repacked, sieved, and undisturbed aquifer sediments. In the field, peak abundance of labeled bacteria traveling laterally with groundwater flow 6 m downgradient from point of injection was coincident with the retarded peak of carboxylated microspheres (retardation factor, RF = 1.7) at the 8.8 m depth, but preceded the bromide peak and the retarded microsphere peak (RF = 1.5) at the 9.0 m depth. At the 9.5 m depth, the bacterial peak was coincident with both the bromide and the microsphere peaks. Although sorption appeared to be a predominant mechanism responsible for immobilization of microbial-sized microspheres in the aquifer, straining appeared to be primarily responsible for their removal in 0.6-m-long columns of repacked, unsieved aquifer sediments. The manner in which the columns were packed also affected optimal size for microsphere transport, which in one experiment was near the size of the small (~2 μm) groundwater protozoa (flagellates). These data suggest that variability in aquifer sediment structure can be important in interpretation of both small-scale field and laboratory experiments examining microbial transport behavior.

## INTRODUCTION

There is an increased interest in understanding the effects of aquifer heterogeneity upon the subsurface transport behavior of bacteria. This is due to the recognized importance of subsurface bacterial mobility in transmission of some waterborne diseases [*Bitton and Harvey*, 1992], in bioresoration of organically contaminated aquifers using nonindigenous or waste-adapted populations [*Wilson et al.*, 1986], in microbially enhanced oil recovery [*MacLeod et al.*, 1988], and in potential migration of genetically engineered bacteria away from subsurface restoration sites to which they will be applied [*Trevors et al.*, 1990]. The literature is replete with field observations of bacterial transport through a variety of granular aquifers (ranging from pebbles to fine sand) over a travel distance of several hundred meters or more [*Dappert*, 1932; *Merrell*, 1967; *Anan'ev and Demin*, 1971; *Kudryavtseva*, 1972; *Martin and Noonon*, 1977; *Sinton*, 1980]. However, lack of information concerning the source terms, hydrologies, and physical variabilities of the investigated systems limits the amount of useful information involving subsurface transport behavior that may be derived from many of the earlier field observations.

Due, in part, to experimental design and interpretational problems associated with physical variability in aquifer structure, most controlled bacterial transport experiments have been restricted to small distances. A number of recent small-scale field and laboratory experiments have compared the transport behavior of various microorganisms in aquifer material with that of the bromide or chloride, which were used as conservative tracers [*Bales et al.*, 1989; *Fontes et al.*, 1991; *Gannon et al.*, 1991; *Harvey et al.*, 1989; *Harvey and Garabedian*, 1991]. Columns packed with aquifer material have been particularly useful for investigating a number of controls on microbial transport behavior, since this allows a much greater control of the variables than is possible in the field [*Harvey*, 1991]. However, even over relatively short distances, heterogeneity in physical structure of an aquifer and differences between aquifer structure in packed columns and the field site from which the aquifer material was collected can limit interpretation of small-scale field and column experiments. Indeed, transport of *Escherichia coli* through repacked soil collected from the unsaturated zone has been demonstrated to be markedly different than that through undisturbed soil [*Smith et al.*, 1985]. Recent bacterial transport experiments involving a layered section of a sandy aquifer [*Harvey and Garabedian*, 1991] and a two-zone sand column [*Fontes et al.*, 1991] suggest that physical variability in aquifer structure increases dissimilarity between bacterial and conservative solute transport behavior in small-scale experiments.

New knowledge is being gained about the effects of aquifer heterogeneity upon the large-scale transport of conservative [*LeBlanc et al.*, 1991; *Garabedian et al.*, 1991; *Hess et al.*, 1992] and reactive contaminant solutes [*Poeter and Gaylord*, 1990; *Barber et al.*, 1992], but there has been little effort to establish the effect of physical (structural) variability on observations of bacterial transport behavior in aquifers. In this study, we investigate the role of variability in the physical properties of aquifer sediments upon microbial transport behavior in small-scale tests. Three types of experiments were performed. In the first experiment, relative transport behavior of bromide, indigenous groundwater bacteria, and bacteria-sized microspheres was compared at different depths in a moderately stratified sandy aquifer. This

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highly permeable, unconfined aquifer, located in Cape Cod, Massachusetts, is thought to be typical of many of the relatively uniform, glaciofluvial, drinking water aquifers in the northeastern United States. In the second experiment, the effect of restructuring aquifer sediments upon transport behavior of three sizes of carboxylated microspheres (0.2–1.3 μm diameters) was assessed by comparing retardation (relative to a bromide tracer) and immobilization in the field to that observed for repacked sediments in 0.6-m-long glass columns. In the third experiment, the effect of heterogeneity among replicate columns of size-fractionated aquifer sediments was assessed using bacteria- and protozoa-sized microspheres. Results of these experiments suggest that physical heterogeneity should be considered in the interpretation of small-scale tests in which the transport behavior of groundwater microorganisms, microbial-sized microspheres, and conservative solutes is compared.

## EXPERIMENTAL METHODS

Effects of aquifer variability among adjacent sandy layers upon transport of bacteria and microspheres relative to bromide were assessed using a small-scale, natural gradient tracer test conducted during May and June 1990. This injection and recovery experiment involved an organically contaminated (1–2 mg/L dissolved organic carbon (DOC)) section of a layered, sandy (~0.5 mm median grain size) aquifer that had been instrumented with a 20 by 220 m array of 15-port, multilevel sampling devices (MLSD) [*LeBlanc et al.*, 1991]. The MLSDs, which were designed to sample discretely at depth along vertical intervals, consist of 15 color-coded, 6.5-mm (diameter) polyethylene tubes encased in 3.2-cm (diameter) polyvinyl chloride (PVC) pipe [*Smith et al.*, 1991]. The polyethylene tubes exit the PVC pipe at fixed intervals (every 0.25 m) and are capped with nylon screens. The instrumented study area is part of the U.S. Geological Survey's Cape Cod Toxic-Substances Hydrology Research Site in Falmouth, Massachusetts, where previous tracer experiments were conducted to assess the applicability of stochastic theory for predictive modeling of conservative solute transport through aquifer sediments over large (>100 m) distances [*Garabedian et al.*, 1991; *LeBlanc et al.*, 1991].

A stainless steel submersible pump was used to collect bacteria-laden groundwater [*Harvey et al.*, 1984] from a screened, PVC (polyvinyl chloride) observation well (5.0 cm diameter, 250 μm slot width) located 100 m downgradient from an on-land, treated-sewage infiltration bed [*LeBlanc*, 1984]. A morphologically diverse population of bacteria was concentrated at the well head from 960 L of contaminated groundwater to 8 L using a hollow-fiber, tangential-flow filtration device [*Kuwabara and Harvey*, 1990] at a processing rate of 2 L/min. Recovery of bacteria in the 8 L of retentate was about 33%. Recovered bacteria (0.2–1.6 μm long) were stained with the fluorochrome DAPI (4′,6-diamidino-2-phenylindole) at 5 μ*M* (final concentration) for 24 hours at 4°C as described by *Harvey et al.* [1989]. DAPI binds specifically to DNA within the cell and, therefore, may not appreciably affect cell surface chemistry, at least over the short term. However, DAPI is known to cause metabolic impairment of laboratory-grown *Escherichia coli* [*Parolin et al.*, 1990], although it is unclear to what extent the indigenous bacteria in very low nutrient groundwater environments may be affected.

Groundwater from the injection depths (8.8 and 9.0 m below surface) was used as the suspending medium for the DAPI-stained bacteria; 0.74-μm (diameter) carboxylated, fluorescent microspheres; and sodium bromide to lessen the degree of chemical changes within the aquifer during the test. The injection depths are in the suboxic (zero to trace amounts of dissolved oxygen), which overlies the mildly reducing, Fe(II)-containing zone deeper in the plume (D. B. Kent, unpublished data, 1991). The *p*H and temperature at the points of injection were 5.7 ± 0.1 and 11.5 ± 0.5°C, respectively. Pretest specific conductance along the interval from 8.8 to 9.5 m below surface varied <1% (from 385 to 386 μS/cm), suggesting that all three levels were clearly within the contaminant plume. Grain size distribution of the aquifer sediments at point of injection is not known, since core samples could not be taken from within the array. However, aquifer sediments sampled earlier near the test site had the following grain size distribution: ~95% of the particulate mass was composed of grains smaller than 2.5 mm, ~80% smaller than 0.90 mm, ~60% smaller than 0.52 mm, ~30% smaller than 0.30 mm, and 10% smaller than 0.21 mm [*Wolf*, 1988]. Final concentrations of bromide, bacteria, and microspheres in the 97 L of injectate were 154 mg/L, 4.30 ± 1.66 × $10^9$/L, and 1.16 ± 0.12 × $10^9$/L, respectively. The injectate was slowly added to the aquifer at well F7-15 at the depths from which the groundwater was collected. Bromide, stained bacteria, and microspheres were monitored at point of injection and over a total horizontal distance of 12 m as they moved with the natural flow of groundwater through rows of MLSD spaced 2 m apart. At 6 m downgradient, the tracer cloud appeared to strike MLSD F10-14 directly (using the tracer cloud configuration assumptions of *Harvey and Garabedian* [1991]). This allowed delineation of the concentration histories for the bromide, bacteria, and microspheres at several depths. Groundwater samples (500 mL) were taken over the time course of tracer breakthrough using new, sample-rinsed polyethylene bottles and stored at 2°–4°C until processed.

Bromide was measured with a specific-ion electrode. Preparations for enumeration of DAPI-stained bacteria and fluorescent microspheres were made with 100–200 mL of sample. Where possible, enough fields were counted so that there were at least 350 stained bacteria enumerated, giving a counting precision of better than ±10%. Due to a greater degree of immobilization within the sediments, counting precision for the resulting lower numbers of microspheres was not as good, but was generally within ±20%. The DAPI-stained bacteria in these samples fluoresced under incident UV light (340–380 nm excitation) and were enumerated on black polycarbonate membrane filters (0.2-μm pore size) using a Leitz microscope that was fitted for epifluorescence [*Harvey et al.*, 1984]. The microspheres (type YG, Polysciences, Warrington, Pennsylvania) fluoresced at a different wavelength than the DAPI-stained bacteria and were enumerated under incident blue light (390–490 nm). The retardation and attenuation in peak concentrations of labeled bacteria, and carboxylated microspheres relative to bromide were compared for the three different levels in the aquifer. Retardation factors were calculated as the velocity ratio of bromide to microspheres or bacteria. Velocities were measured for peak abundances at the MLSD 6 m downgradient from point of injection.

To examine the effect of pore structure alterations upon transport characteristics of bacteria-sized particles, aquifer

sediments were collected near the point of injection (~9 m below surface) at the site of a previously published small-scale transport test involving bacteria and bacteria-sized microspheres (site F347) [*Harvey et al.*, 1989; *Harvey and Garabedian*, 1991], heat sterilized, and repacked into glass columns (0.6 m long, 5.0 cm ID). The columns were packed as uniformly as possible using a mechanical packer (Soil Physics Lab, U.S. Geological Survey, Menlo Park, California). Much of the stratigraphy evident in cores taken from the saturated zone and in vertical cuts through the unsaturated zone at the Cape Cod site was destroyed in the mechanical repacking process. The packed column had a porosity of ~0.35, which was similar to the average porosity calculated for the aquifer in the area of the tracer test site [*LeBlanc*, 1984]. Columns were initially eluted with filter-sterilized $CO_2$, followed by degassed 0.005 *M* $CaSO_4$ solution. Flow-through column experiments were then conducted in the upflow mode at 20°C and 40 mL/h (1.4 m/d linear velocity) using an 0.5-L reservoir-type, syringe pump (ISCO model 5000) that delivers accurately at low flow rates. Well-characterized, bacteria-sized microspheres (carboxylated latex; 0.2, 0.7, and 1.35 $\mu$m diameter) and bromide (20 mg/L) were added as a continuous injection to the columns. A lower concentration of bromide was used in the column tests relative to what was used in the field, because of the lower degree of dilution with bromide-free water. Immobilization and retardation for the different sizes of carboxylated microspheres were compared to data from a small-scale, forced-gradient field test reported earlier [*Harvey et al.*, 1989].

A second column experiment was conducted in order to assess the effect of sediment packing variability upon the relative transport behavior of bacteria- and protozoa-sized, carboxylated microspheres (0.46, 0.72, 1.7, 2.8, and 4.8 $\mu$m diameter). In this experiment, two glass columns were packed with the 0.5–1.0 mm size fraction of Cape Cod subsurface sediments. Both columns were prepared using a wet packing procedure described by *Johnson* [1990] and run in an upflow mode (10° from horizontal) at 10°C using a 4-L storage reservoir followed by a 0.5-L inverted siphon (Mariot) reservoir to maintain a constant head. Contents of the two reservoirs were continuously stirred. Samples of effluent were collected in 22-mL glass scintillation bottles using an Eldex (San Carlos, California) model U1A fraction collector. Flow rate through the column and dimensionless (calculated as the ratio of effluent to influent) concentrations for bromide and the five sizes of microspheres were monitored in each column. Comparisons were then made between the rates of retardation and immobilization among the four classes of microspheres. Microsphere size classes were differentiated under the microscope on the basis of size and fluorescence; the 0.45-, 1.7-, and 4.8-$\mu$m (diameter) spheres were type BB (emission maximum at 468 nm; excitation maximum at 365 nm) and fluoresced under incident UV light, whereas the 0.72- and 2.82-$\mu$m spheres were type YG (emission maximum at 540 nm; excitation maximum at 458 nm) and fluoresced under incident blue light.

## RESULTS

### *Field Experiment*

Breakthrough curves for the DAPI-stained indigenous bacteria, the 0.74-$\mu$m (diameter) carboxylated microspheres, and bromide at 6 m downgradient from point of injection (MLSD 10-14) in the large-scale array are depicted in Figure 1 for three layers of aquifer sediments at 8.8, 9.0, and 9.5 m below surface. The relationship among the concentration histories of bacteria, microspheres, and bromide varied markedly with depth. At 8.8 m below surface, the peak in bromide concentration clearly preceded the peaks in labeled bacteria and microspheres and there appeared to be little overlap in breakthrough curves for bromide and those for the unattached bacteria or the microspheres. The breakthrough curve for the microspheres exhibited a single peak that was coincident with the retarded peak in bacterial abundance. Microscopic observations revealed that the bacteria were not physically associated with the microspheres. At 9.0 m below surface the bromide and bacterial breakthrough patterns were quite similar, both exhibiting coincident, single peaks. However, the microspheres were clearly retarded with respect to both bacteria and bromide. Finally, at 9.5 m below surface the breakthrough curves for all three constituents exhibited single peaks that were coincident and similar in shape.

Retardation of microspheres and bacteria with respect to bromide at MLSD 10-14 are summarized in Table 1 for the three depths. The retardation factor (RF) for peak abundance of carboxylated microspheres at 8.8 m below surface (1.7) was somewhat higher than that observed by *Harvey et al.* [1989] (RF 1.4) for 0.2–0.9 $\mu$m carboxylated microspheres in a previous small-scale experiment at nearby site F347. At 9.0 m below surface the retardation factor for the microspheres (1.5) was closer to what was observed earlier at the F347 test site. However, little retardation of carboxylated microspheres was observed for the 9.5 m depth, in contrast to all earlier observations made at the Cape Cod site. Retardation factors for the bacteria ranged from 0.8 at 9.0 m below surface to 1.7 at 8.8 m below surface. The earlier arrival in peak bacterial abundance with respect to bromide was consistent with an earlier observation involving a small-scale, forced-gradient experiment in another area of the aquifer (site F393) [*Harvey et al.*, 1989], although coincident peak breakthrough of bromide and labeled bacteria was observed in the site F347 experiment [*Harvey and Garabedian*, 1991]. Retardation of labeled bacteria with respect to bromide (8.8 m depth, this experiment) had not been observed in previous small-scale transport studies at the Cape Cod site.

### *Column Experiments*

A field versus column comparison of transport breakthrough parameters for small-scale experiments involving microbial-sized, carboxylated microspheres is given in Table 2. In the column experiment, maximum dimensionless concentrations for the microspheres were directly related to the mean diameter of the size class, i.e., smaller microspheres were attenuated by the media to a lesser degree than larger microspheres. The largest (1.3 $\mu$m) size class was subject to a seventeenfold higher rate of immobilization than was the smallest (0.2 $\mu$m) size class. This was in direct contrast to their transport behavior in an earlier field experiment [*Harvey et al.*, 1989], where the smaller microspheres were immobilized in aquifer sediments to a greater degree than were the larger microspheres (Table 2).

Retardation factors for the field and column experiments

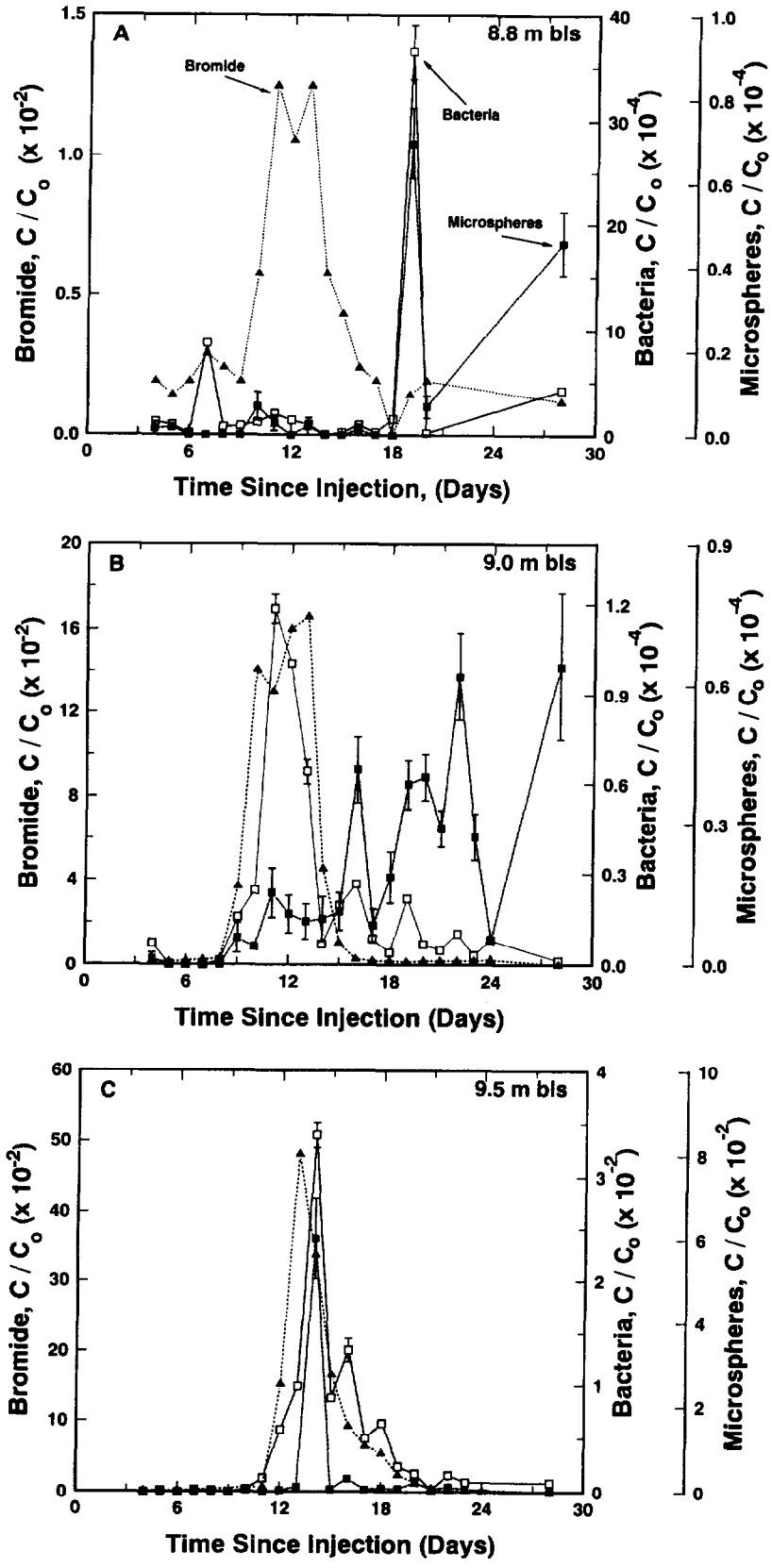


Fig. 1. Concentration histories for labeled indigenous bacteria (solid squares), 0.74-$\mu$m (diameter) carboxylated microspheres (open squares), and bromide (triangles) at well M10-14, 6 m downgradient from point of injection in a natural gradient test involving a sandy aquifer in Falmouth, Massachusetts. Breakthrough curves are for the (*a*) 8.8 m depth, (*b*) 9.0 m depth, and (*c*) 9.5 m depth.

TABLE 1. Retardation and Maximum Dimensionless Concentrations for 0.74-μm (Diameter) Carboxylated Microspheres, Labeled Indigenous Bacteria, and Bromide

| Depth, m | Conditions[a] | | Colloid | Retardation Factor | $(C/C_0)_{max} \times 10^{-2}$ |
|---|---|---|---|---|---|
| | pH | TDS, mg/L | | | |
| 8.8 | 5.8 | 181 | microspheres | 1.7 | 0.007 |
| | | | bacteria | 1.7 | 0.37 |
| | | | bromide | ··· | 1.25 |
| 9.0 | 5.8 | 186 | microspheres | 1.5 | 0.006 |
| | | | bacteria | 0.8 | 1.19 |
| | | | bromide | ··· | 16.6 |
| 9.5 | 5.9 | 197 | microspheres | ~1.0 | 0.06 |
| | | | bacteria | ~1.0 | 3.39 |
| | | | bromide | ··· | 48.2 |

Data are from the natural gradient test at U.S. Geological Survey well M10-14, 6 m downgradient from point of injection.

[a]Values are for well 10-14 during breakthrough of bromide. The pH and total dissolved solids (TDS) were measured on day 11 and day 21, respectively. Initial conditions at comparable depth at the point of injection (well 7–15) were 5.7–5.8 (pH), 12°C (temperature), 385–387 μS/cm (specific conductivity).

are also shown in Table 2 for three size classes of microspheres. For the column experiment, retardation factors of each size of microsphere were substantially less than unity, ranging from 0.53 for the smallest microspheres to 0.63 for the largest. This suggests that the microspheres have, on the average, taken a more direct path of travel through the column than the conservative solute. However, substantial retardation of all three size classes of microspheres relative to bromide was observed in the field [*Harvey et al.*, 1989]. Retardation factors ranged from 1.1 for the largest size class to 1.4 for the smallest.

Breakthrough curves for the 0.45-, 0.72-, 1.7-, 2.8-, and 4.8-μm (diameter) microspheres for each of two replicate 0.6-m glass columns packed in a similar manner with sieved (0.5–1.0 mm grain size) subsurface sand are depicted in Figures 2*a* and 2*b*. Physicochemical conditions in the feed water for the two were similar (7.0 ± 0.1 pH, 10°C, and ~150 mg/L total dissolved solids). For both saturated sand columns, peak breakthrough of the various size classes of carboxylated microspheres was retarded relative to bromide and breakthrough of the largest microspheres (4.8 μm) was insignificant. However, the two columns differed significantly in the relative transport behavior of the other size classes of microspheres. While few 2.8-μm (diameter) microspheres were evident in column A effluent, dimensionless concentration of this size class in the effluent of column B ranged up to ~2%. The largest difference in relative transport behavior for the two column runs was evident for the three smallest size classes of microspheres (0.45, 0.72, and 1.7 μm diameters). In column A, differences between the breakthrough curves for these size classes were not clear for the last half of the run. In contrast, fractional breakthrough of the 1.7-μm (diameter) microspheres in column B was generally fivefold greater than for the 0.45- and 0.72-μm microspheres over the same time period.

## DISCUSSION

### *Effects on Retardation*

The effect of aquifer variability on retardation of carboxylated microspheres was quite evident from Figure 1, which shows the patterns of breakthrough for three adjacent layers of aquifer sediment (8.8, 9.0, and 9.5 m below surface).

TABLE 2. Retardation Factors and Maximum Dimensionless Concentrations for Breakthrough of Selected Carboxylated Microspheres

| Microsphere Diameter, μm | $(C/C_0)_{max} \times 10^{-4}$ | | | Retardation Factor | |
|---|---|---|---|---|---|
| | Field[a] | Column | | Field[a] | Column (Unsieved)[d] |
| | | Unsieved[b] | Sieved[c] | | |
| 0.2 | 0.06 | 3.03 | ··· | 1.4 | 0.53 |
| 0.5 | 0.44 | ··· | 34–167 | 1.4 | |
| 0.7 | ··· | 0.29 | 74–115 | na | 0.57 |
| 1.3 | 0.65 | 0.18 | ··· | 1.1 | 0.63 |
| 1.7 | ··· | ··· | 81–747 | na | |
| 2.8 | ··· | ··· | 17–141 | na | |
| 4.8 | ··· | ··· | 00–00 | na | |

Field data are from 6.9 m downgradient from point of injection at well site F347 (Falmouth, Massachusetts), and column data are from 0.6-m-long columns of repacked or sieved aquifer sediments from the field site. Here, na denotes not applicable.

[a]Field data from *Harvey et al.* [1989]; pH was 5.8.

[b]The pH was 5.5.

[c]The pH was 7.0; grain size: 0.5–1.0 mm.

[d]Retardation could not be accurately determined for the sieved sediment columns.

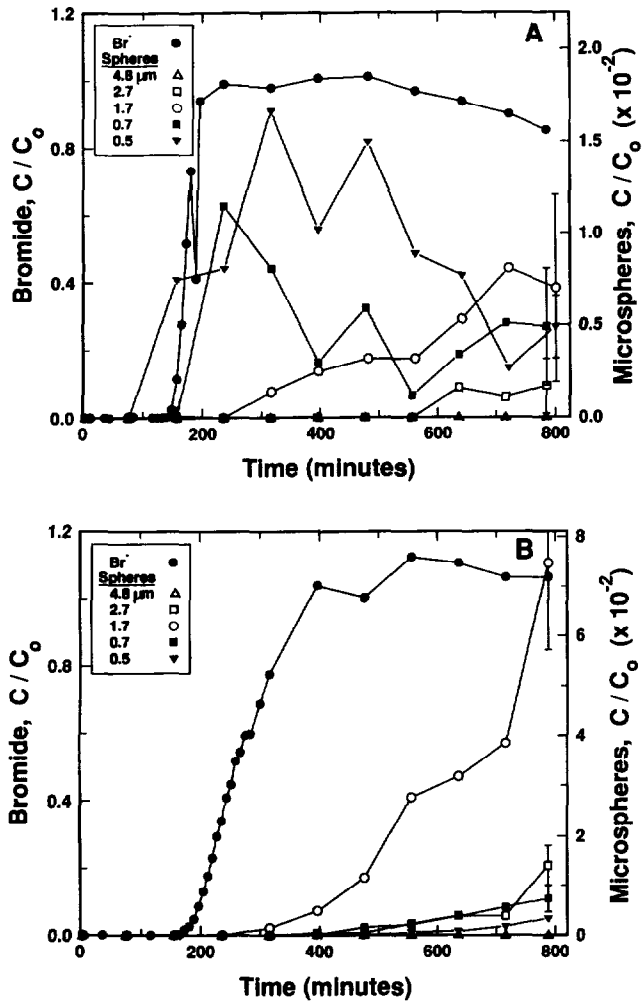


Fig. 2. Dimensionless concentration histories, ($C/C_0$) versus time, for bromide and bacterial- and protozoan-sized carboxylated microspheres (0.45, 0.72, 1.7, 2.8, and 4.8 $\mu$m diameter) in the effluent of 0.6-m-long columns packed with the 0.5–1 mm grain size fraction of subsurface sand from the Cape Cod groundwater study site. Results are depicted for replicate columns: (*a*) column A and (*b*) column B. The *p*H in both experiments was 7.0 ± 0.1.

Indeed, the order in which peak abundances of labeled bacteria, microspheres, and bromide appeared downgradient at well M10-14 differed among the three depths. Earlier appearance of the bacterial peak relative to bromide (9.0 m below surface, Figure 1*b*) had been observed in a previous forced-gradient experiment performed at another location in the same aquifer [*Harvey et al.*, 1989]. Earlier downgradient arrival relative to conservative tracers has been reported for transport experiments involving saturated sandy sediments and yeasts [*Wood and Ehrlich*, 1978], viruses [*Grondin and Gerba*, 1986], iron oxide colloids [*Puls and Powell*, 1992], and even high–molecular weight macromolecules such as dextran [*Enfield and Bengtsson*, 1988]. Rapid migration of microorganisms relative to conservative tracers is believed to be due to microbal movement along preferred flow paths or exclusion from a fraction of the total porosity [*Bitton and Harvey*, 1992]. It is not clear which mechanism is responsible for the apparent acceleration of bacterial transport within certain sections of Cape Cod aquifer sediments. Substantive differences in hydraulic conductivity and macrodispersion along 15-cm intervals on vertical transects through the Cape Cod aquifer have been reported by *Hess* [1989]. The data suggest the presence of narrow (less than 1 m thick) zones of similar conductive properties that persist for several meters horizontally in the region of the small-scale bacteria and microsphere transport experiments. Bromide traveling through a more transmissive zone would be expected to diffuse into adjacent lenses of fines to a greater extent than would the stained bacteria, which would cause a longer average path of travel for bromide.

Intragranular diffusion of bromide into internal porosity too small to permit bacterial entry may also contribute to the phenomenon of relatively accelerated bacterial transport at the Cape Cod site. It has been reported that there is internal porosity within feldspar, quartz, biotite, and muscovite grains collected from the subsurface at the Cape Cod site [*Wood et al.*, 1990]. Internal porosity within grains of quartz and feldspar, which constitute the bulk of the sediment mass within the aquifer [*Barber et al.*, 1992], averaged 11 and 9%, respectively. However, it is not known how the internal porosity of the grains varies from layer to layer within the aquifer sediments at Cape Cod as a result of spatial variability in mineralogy. Substantive spatial variations in the distribution of grains with internal porosity may help explain why earlier arrival of peak abundance of labeled bacteria relative to bromide is only observed for certain depths and locations at the Cape Cod site.

Later arrival of the peak in bacterial abundance relative to the bromide peak likely involves a very different mechanism. Although pore water chemistry varied little among the three depths, there may have been some effect caused by stratigraphic differences in mineralogy. In recent laboratory and field experiments, it was observed that substantial numbers of DAPI-stained bacteria added to Cape Cod aquifer sediments sorb reversibly to the sediment grains [*Scholl and Harvey*, 1992]. Sorption reversibility, a major control of bacterial retardation within the aquifer, was further found to be altered by the presence of metal (iron, manganese, and aluminum) oxides and organic material on grain surfaces, as well as dissolved organic contaminants within the plume. Spatial variability in site mineralogy as it relates to the sorptive behavior of organic solutes on a larger (plume-wide) scale is described by *Barber et al.* [1992]. Adsorption of several organic contaminants within the plume was shown in the latter study to be controlled to a large degree by iron-rich minerals, which are most abundant in finer-grained aquifer sediments. Therefore retardation of organic contaminants and possibly bacteria may be enhanced in layers or pockets of fine-grained sediments. Heterogeneity in the distribution of minerals, particularly those that are primarily responsible for the reversible sorption of bacteria, likely contributes to the differences in bacterial retardation that were observed for the labeled bacteria for the 8.8 and 9.5 m depths (RF ~1 versus 1.7, respectively (Figures 1*a* and 1*c*)).

The fact that the peak in bacterial abundance elutes coincidentally with that of the microspheres at two of the depths (8.8 and 9.5 m below surface), but substantively precedes the microsphere peak at 9.0 m below surface is more difficult to explain. It is possible that the controls that influence apparent velocity relative to a conservative tracer (e.g., the degree of exclusion from internal porosity, the distribution of intergranular pore sizes, and the quantity and type of grain surfaces at which colloidal material reversibly

adsorbs) may act differentially upon the bacteria and bacteria-sized microspheres. Differences in morphology and surface characteristics between the largely rod-shaped bacteria and the carboxylated microspheres undoubtedly play a role. In general, interaction with stationary surfaces in the aquifer appears to be a more important factor in the transport behavior of microspheres in aquifer sediments than of bacteria. This may explain the microspheres' 2–order of magnitude greater immobilization relative to bacteria in this study and in earlier experiments [*Harvey et al.*, 1989]. The degree of retardation of bacteria-sized microspheres appears to increase with the reactivity of their surfaces; in an earlier experiment in which retardation in Cape Cod aquifer sediments was compared for similar-sized neutral, carbonyl, and carboxylated microspheres, the more reactive carboxylated microspheres were retarded the most [*Harvey et al.*, 1989]. Also, apparent dispersion of the carboxylated microspheres was much greater than that observed for labeled bacteria of similar size and may be more sensitive to the effects caused by spatial variability in mineralogy.

The more rapid transport of the microspheres relative to bromide in the column experiment involving whole aquifer sediments (Table 2) suggests that substantial secondary pore structure was created when the column was dry packed with aquifer material or when it was resaturated with water. It does not appear that there is enough internal porosity within the grains to account for the lag in peak bromide arrival in the column effluent, even if bromide could access all of the internal porosity. It has been observed, from previous flow-through column experiments (R. W. Harvey, unpublished data, 1988) that transport characteristics of bacteria and microspheres relative to those of a conservative tracer are, in part, dependent upon the manner in which the columns are packed. Although use of the vibration-free column packer has been demonstrated to reduce mechanically induced heterogeneity in previous soil column experiments [*Ripple et al.*, 1974], uniform packing with whole heterogeneous sediments from the aquifer into large columns appears to be problematic. It is clear that the effect of pore structure rearrangement in packed columns upon the apparent retardation of microorganisms or microspheres relative to conservative tracers cannot be overlooked.

### *Effect on Immobilization and Optimal Size for Transport*

The rate of bacterial immobilization with respect to bromide differed among the three sampled depths in the natural gradient experiment reported on herein (Table 1) and among the two sampled depths in a previous experiment at nearby well site F347 [*Harvey and Garabedian*, 1991]. Immobilization of bacteria in aquifer sediments at the Cape Cod site likely depends upon a number of factors, including surface and groundwater chemistries [*Scholl and Harvey*, 1992], cell size [*Harvey et al.*, 1989], cell morphology, and grain size distribution. Levels of growth-stimulating nutrients can also affect the propensity of bacteria for solid surfaces [*Kjelleberg*, 1984]. Within the contaminant plume at the Cape Cod site, partitioning of bacteria to the solid phase increases with increasing distance downgradient (and with the age of the DOC) [*Harvey and Barber*, 1992]. However, differences in groundwater chemistry between the three monitored depths in the in situ transport experiment at M10-14 appear to be minimal. In contrast, there is no evidence that the chemistry of the grain surfaces was similar from depth to depth. Core material was not taken within the test array, due to potentially adverse consequences upon future tracer tests at that site. However, subsurface sediments in the sampling array have been shown to be highly stratified [*LeBlanc et al.*, 1991] and subject to heterogeneity in particle size distribution and mineralogy [*Barber*, 1990; *Barber et al.*, 1992].

From Table 2 it is clear that the optimal size for microbial transport depends upon the manner in which the various sized grains are arranged within the aquifer sediments. For well-sorted, undisturbed aquifer sediments, attenuation of the bacteria-sized microspheres was inversely related to size, i.e., the smallest microspheres were sorbed from moving groundwater to a greater degree. This observation is consistent with colloid filtration theory, which predicts that smaller particles in the size classes of groundwater bacteria (0.1–1 $\mu$m) being transported through porous media become immobilized faster than larger bacteria–sized particles. This is because nonadvective particle movement in this size range and the likelihood that colloids contact stationary surface are governed largely by diffusion, which increases with decreasing colloidal size. Differences in the degree of immobilization among microsphere size classes agreed closely with what was predicted using an advection-dispersion-filtration model [*Harvey and Garabedian*, 1991].

Straining should not be much of a factor in undisturbed aquifer sediments from the site, assuming grain size distributions comparable to the ranges reported by *Barber* [1990]. Theoretically, straining should only be significant if the ratio of microsphere diameter ($d_{ms}$) to the critical pore size is greater than 1.5 [*Matthess and Pekdeger*, 1985], i.e.,

$$\frac{d_{ms}}{F_s d_k} \geq 1.5 \qquad (1)$$

where $F_s$ is the empirical transit factor for suffusion and is related to the heterogeneity of the porous media and $d_K$ is the hydraulic equivalent diameter of pore canals. The latter parameter is equivalent to 0.455 $U^{1/6} e d_{17}$, where $U$ is the uniformity coefficient (calculated as $d_{60}/d_{10}$) and $e$ is the ratio of void to solid volume in the media. The parameters $d_{17}$, $d_{60}$, and $d_{10}$ are the grain diameters at which 17, 60, and 10%, respectively, of the particulate mass is of a smaller size. In general, the ratios of the microsphere diameter to the critical pore size in Cape Cod aquifer sediments are well below 1.5.

That immobilization of microspheres in the column of repacked aquifer sediment was directly related to size (Table 2), in contrast to what was observed in the field, suggests that straining may have been more significant in the repacked sediments. Visual inspection of the column suggested that a portion of the fine material had migrated to one end of the column, causing nonhomogeneity in the distribution of grain sizes. The finer-grained material at one end of the column may have facilitated preferential removal of the larger microspheres in that zone. This may also help explain the higher rates of immobilization (per unit of advective travel) in the columns relative to the field.

It is not known how much of the spatial fractionation of grain sizes occurred during the dry mechanical packing operation or during column rewetting. However, it is clear that the repacking of whole aquifer sediments into columns may have significant ramifications with regard to microbial

transport behavior, particularly in experiments requiring large columns. In several recent laboratory experiments involving bacterial transport through saturated media [*Lindqvist and Bengtsson*, 1991; *Fontes et al.*, 1991; *Scholl and Harvey*, 1992], problems associated with fractionation of grain size fractions have been ameliorated by the use of smaller-diameter, shorter-length columns packed with sieved sand. However, the effect of macropore (secondary pore structure) destruction and sieving upon transport of bacteria in unsaturated soil columns has been clearly documented [*Smith et al.*, 1985]. Our data suggest substantial alterations in bacterial transport may also result from mechanical disruption of intergranular pore structure within whole aquifer sediments. Therefore smaller columns of sieved material may be more useful for examining specific controls of subsurface transport behavior of microorganisms.

The optimal size for transport of carboxylated microspheres (specific gravity, 1.05) through 0.5–1.0 mm (grain size) aquifer sediments (Figure 2*a*) was found to be close to the size class of the smaller protozoa found in the aquifer sediments at the Cape Cod site, i.e., ~2 $\mu$m (diameter) [*Kinner et al.*, 1992]. This can be predicted by the theoretical minimum in the so-called collector efficiency of the porous medium [*Rajagopalan and Tien*, 1976]:

$$\eta = 0.72 A_s N_{Lo}^{1/8} N_R^{15/8} + 0.0024 A_s N_G^{1.2} N_R^{-0.4} + 4 A_s^{1/3} N_{Pe}^{-2/3}$$

where $A_s = 2(1 - p^5)/w$; $w = 2 - 3p + 3p^5 - 2p^6$; $p = (1 - \theta)^{1/3}$; $N_{Lo} = H/9\pi\mu a_p^2 V$; $N_R = a_p/a_s$; $N_G = 2a_p^2(\rho_p - \rho)g/9\mu U$; and $N_{Pe} = 2Va_s/D$. In the above expressions, $a_p$ and $a_s$ are the respective colloid and grain radii, $\mu$ is the absolute viscosity, $V$ is the approach velocity, $\theta$ is the external porosity, $\rho$ and $\rho_p$ are the respective densities of water and the colloidal particle, $g$ is the acceleration due to gravity, and $H$ is the Hamaker constant. The optimal size for microbial transport in a porous medium depends, in part, upon the buoyant density (specific gravity) of the microbe. In general, the lower the specific gravity (more neutrally buoyant), the larger the optimal size for transport becomes [*Harvey*, 1991]. For carboxylated microspheres (1.05 g/cm$^3$), minimal attenuation should occur for diameters between 1 and 2 $\mu$m, which is consistent with what we observed for one of the two columns packed with 0.5–1.0 mm (grain diameter) aquifer sediments (Figure 2*b*).

The reasons for the apparent differences in the optimal size for microsphere transport for column A (Figure 2*a*) and column B (Figure 2*b*) are not clear. Deviations from theoretical predictions of microsphere immobilization moving through packed porous media columns have been observed even for uniform, well-defined, spherical collectors (glass beads) [*Tobiason and O'Melia*, 1988]. It was hypothesized that such deviations may be due, in part, to the effects of surface roughness, heterogeneity among the population of collector surfaces and suspended particles, possible inadequacies of the equilibrium DLVO (Derjaguin-Landau and Verwey-Overbeek) theory of colloid stability [*Shaw*, 1976] and non-DLVO forces. It was also observed that the degree of microsphere immobilization in their column experiments was extremely sensitive to changes in solution chemistry. In our column experiments, apparent differences in relative transport behavior among the two columns may have resulted from differences in the distribution of heterogeneous sand grains or areas of desaturation, even though efforts were made to use the same sieved aquifer sediments and packing techniques in each column.

In summary, it appears likely that physical heterogeneity within sandy aquifer sediments can have a very significant effect on the transport behavior of microorganisms being transported with the groundwater. Although the effects upon microbial transport behavior that are caused by changes in grain size distribution and changes in mineralogy can be difficult to sort out, it does appear that the transport of well-characterized microbial-sized particles relative to that of a conservative tracer can be substantively different in aquifer sediment in which the pore structure has been altered. Alterations in grain size distribution of sandy aquifer sediments can substantively affect relative contributions of straining and sorption in observations of colloid or microbe immobilization in the lab. Also, physical variability over relatively short distances (within a meter on the vertical scale) even in relatively uniform aquifers can lead to substantive changes in apparent dispersion of and relative retardation between microorganisms, microbial-sized microspheres, and conservative tracers in small-scale tests. Finally, it appears that a great deal of caution must be exercised when extrapolating results of column studies of microbial transport to groundwater environments, since alterations in transport behavior which occur as a result of repacking aquifer sediments into flow-through columns can be quite complex. It is clear that modeling efforts to describe microbial transport in sandy aquifer sediments may benefit from additional information on the role of physical variability on the controls of microbial transport and the scale at which the effects of this variability even out.

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