Pore-Scale Quantification of Colloid Transport in Saturated Porous Media

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It is currently not clear how to quantifiably relate pore-scale observations of colloid transport to larger scales, so, we proposed a geometric theory showing that pore-scale-derived rate constants may be appropriate to model a larger scale system. This study considered three different types of colloids: latex microspheres, *Escherichia coli*, and microspheres made of poly lactic acid (PLA). Colloid attachment and detachment rate constants were calculated using digital microscope images, taken in rapid (1 s) sequences, from which rates of attaching and detaching colloids were readily observed. Average rate constants from >1000 images per colloid-type were used to model Darcy-scale colloid transport breakthrough curves. The modeled and observed breakthrough curves agreed well for all three types of colloids. However, for latex and PLA microspheres, the model systematically under predicted the breakthrough curves’ rising limb, which may indicate that the rate “constants” are actually dependent on the amount of attached colloids. Insights into these sorts of complexities are best addressed by research that considers both pore-scale phenomena and larger-scale transport responses.

Introduction

There is increasing concern about subsurface transport of colloidal-sized materials, diameter of 1 nm to 10 μm, especially pathogenic biocolloids and abiotic materials to which normally immobile contaminants may attach and be transported (1). Colloid transport mechanisms are also important for bioaugmentation and biofouling during bioremediation. The ability to predict colloid transport is still not fully realized, especially with respect to the quantification of colloidal reaction rates during transport through porous media. Most previous studies have been based on mass balance and breakthrough curves in packed sand columns (2–5); as such, predictions of tracer movement have been limited to “black box” modeling. Several researchers have used pore-scale visualization techniques to gain insight on mechanisms that would be difficult to observe in the environment (6–10). These visualization studies have provided valuable qualitative information about features of transport mechanisms, e.g., straining, grain retention, retention in immobile pore spaces, and air/water/surface interface interactions. Only a few studies have used pore-scale visualizations to describe colloid transport quantitatively (6). There remains a critical knowledge gap in quantifying the observed pore-scale mechanisms to large-scale colloid transport behavior.

The convective-diffusion equation for colloids in saturated media can be expressed as

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - \frac{\rho_b \partial S}{\partial t}
\]

(1)

where \( C \) is the concentration of suspended colloids (mg L\(^{-1}\)), \( D \) is the diffusion or hydrodynamic dispersion coefficient (m\(^2\) h\(^{-1}\)), \( v \) is the average linear water velocity (m h\(^{-1}\)), \( x \) is the travel distance in the direction of flow (m), \( \rho_b \) is the media bulk density (g L\(^{-1}\)), \( n \) is the porosity, and \( S \) is the deposited/retained colloid concentration (mg colloid per g sand). The last term includes both the rate of colloid retention and remobilization. Colloid transport studies have generally only considered bulk attachment, modeling the process with first order irreversible kinetics. This is due in part from the classic filtration theory first developed by Yao et al. (11), which provides an easy way to calculate colloid transport rate in porous media (12). The clean bed filtration theory works fine for the idealized experimental systems (i.e., columns packed with uniform sized glass beads), however, several studies indicate that the theory needs to be modified to apply to the real porous medium such as quartz sand (e.g., refs 13 and 14). More recent studies have shown that both attachment and detachment processes are significant in colloid transport (3, 4). The colloid deposition term can be expressed as

\[
\frac{\partial S}{\partial t} = \frac{n}{\rho_b} C - k_d S
\]

(2)

where \( k_a \) is the first-order attachment rate constant (h\(^{-1}\)) and \( k_d \) is the first-order detachment rate constant (h\(^{-1}\)). In previous studies, \( k_a \) and \( k_d \) have been calibrated to best-fit these equations to breakthrough-curve data (3, 4). However, with any optimized modeling investigation involving several parameters, similarly good fits can be achieved with more than one combination of variables.

In this study, we determined colloid breakthrough curves from a flow chamber and also visualized colloid transport at the pore scale via rapid (1 s) digital image sequences. Our main objective was to mechanistically model Darcy-scale colloid transport using independently estimated, pore-scale attachment and detachment rate constants. Another objective was to compare the transport behavior of three types of colloids: carboxylated latex microspheres, *Escherichia coli*, and poly(\(-\)lactide) (PLA) microspheres. The latter colloid type, PLA microsphere, was chosen because we believe it may serve as an ideal colloidal tracer at landscape scales since it can encapsulate unique labels (e.g., DNA or nanobarcodes) to uniquely distinguish one sphere from another, thus allowing researchers to simultaneously distinguish among several interacting tracer signals. Unlike commonly used latex microspheres, PLA is degradable (nonpolluting), biocompatible, and nontoxic both as a polymer and degradation products (15). In fact, PLA has been widely investigated for medical applications, e.g., dissolvable sutures and slow...

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release drugs and other chemicals, and as such, has been approved by the FDA for human clinical applications. Because PLA has been so widely used in medicine, we can build on copious experience in fabricating PLA microspheres with specific properties (16, 17). The only environmental application of degradable microspheres to date is the work by Quaglia et al. (18), who used microspheres composed of mono-, di-, and triglycerides encapsulating a pesticide as a novel method for application of agrochemicals.

Theory. It is currently unclear how to quantifiably relate pore-scale observations to larger scale systems. As a first approximation, we provide the following logic to suggest that attachment and detachment rates observed at the pore-scale may be appropriate for describing Darcy-scale behavior. At the pore-scale, we view the system through a microscope as a plane in which we can distinguish among pore, soil grain, and colloid areas, \( A_p, A_g, \) and \( A_c \), respectively. Since we are focusing on saturated soils, all pore area is filled with water and colloids. We can translate \( A_p \) and \( A_g \) into volumes by multiplying by the focal depth (\( d \)). Assuming the colloids are spherical with radius, \( r \), we can translate \( A_c \) into a volume by multiplying by \( 4r/3 \). Now, \( C, S, \) and \( n \) in eqs 1 and 2 can be written as

\[
S = \frac{\rho_c A_g A_c^{4/3} r}{\rho_g A_p d} \tag{3}
\]

\[
C = \frac{\rho_c A_g A_c^{4/3} r}{A_p d} \tag{4}
\]

\[
n = \frac{A_c d}{A_p d} \tag{5}
\]

where \( \rho_c \) is the density a colloid (\( \text{mg m}^{-3} \)), \( \rho_g \) is the density of the soil grain (\( \text{mg m}^{-3} \)), \( A_g \) is the area of colloids suspended in the pore space as seen in the microscope images (\( m^2 \)), and \( A_c \) is the area of colloids attached on the grain in the microscope images (\( m^2 \)). We can convert bulk density into a two-dimensional parameter via \( A_p/A_T \) (\( A_T \) is the total image area (\( m^2 \)), \( A_p + A_g + A_c \)). Substituting eqs 3–5 and \( \rho_c = \rho_g/(A_p/A_T) \) into eq 2 and diving through by the mass of an individual colloid, reduces it to

\[
\frac{\Delta N_c}{\Delta t} = k_c N_c - k_d N_g \tag{6}
\]

where “\( N_g \)” and “\( N_p \)” are a “colloid counts” for the parts of an image associated with the soil grain surface and the pore space, respectively and \( \Delta t \) is the time between two observations (microscope images). Note, the rate constants \( k_c \) and \( k_d \) for the two-dimensional system are, at least theoretically, the same as \( k_c \) and \( k_d \) for the three-dimensional system, respectively.

Materials and Methods

Experimental Apparatus. Colloids investigated were carboxylated latex fluorescent microspheres (Magsphere, Pasadena, CA, emission = 488 nm, excitation = 528), genetically modified *Escherichia coli* K-12 PHIL 628 that constitutively expressed a green fluorescent protein (emission = 488 nm, excitation = 528 nm) (20, 21), and PLA microspheres (16, 22–24). Colloid properties are summarized in Table 1 and additional details on colloid preparation can be found in the Supporting Information. All experiments used a horizontal flow cell (Figure 1) packed with silica sand (300–600 \( \mu \text{m}, \) Unimin Co., NJ) acid washed in 50% nitric acid, rinsed in deionized water, and subsequently dried at 105 °C for 24 h (19). A ceramic porous plate (RH 1000-course pores, R&H Filter Co.) was placed on each end of the column to evenly distribute flow. A syringe pump provided steady influent flow rates (0.15 mL min\(^{-1}\), \( v = 116 \text{ cm h}^{-1} \)) and a peristaltic pump operating at a higher rate was used to remove effluent from the downstream end of the flow chamber. To stabilize pH in the system, all colloids were suspended in phosphate buffer solution (PBS: 0.26 g KH₂PO₄, 1.156 g Na₂HPO₄ per L Milli Q, pH 7.4, ionic strength = 23 mM). PBS and sand were sterilized via autoclaving for bacteria and PLA experiments. A conservative tracer (0.1 M NaCl) was used to estimate the dispersion coefficient, and verify the calculated average flow velocity and sand porosity.

![FIGURE 1. Schematic of flow chamber; dimensions = 4.68 × 1.15 × 0.3 cm; sand porosity, \( n_s = 0.34 \); flow rate = 0.15 mL min\(^{-1}\).](image)

![FIGURE 2. Schematic showing how attachment and detachment rates were calculated based on images taken once per second. The dark circles indicate colloids. The first row represents the original data. The second row shows how many colloids were attached between two consecutive images. As indicated in the third row, the change in the number of attached colloids between images was used to determine the attachment and detachment rates constant. This method is similar to that presented in ref 22.](image)

**TABLE 1. Physical and Chemical Properties of Sand and Colloids.** Parentheses show standard deviations on four samples for zeta potential and the octanol–water partitioning coefficient (**K**\(_{ow}\))

<table>
<thead>
<tr>
<th>Material</th>
<th>Diameter (( \mu \text{m} ))</th>
<th>Zeta Potential (mV)</th>
<th><strong>K</strong>(_{ow})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>300–600</td>
<td>-58.9 (4)</td>
<td></td>
</tr>
<tr>
<td>Latex microspheres</td>
<td>1.1</td>
<td>-65.9 (3.6)</td>
<td>0.64 (0.10)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>1.0 × 1.5</td>
<td>-40.4 (5.0)</td>
<td></td>
</tr>
<tr>
<td>PLA microspheres</td>
<td>1.04 (0.4)</td>
<td>-36.7 (4.3)</td>
<td>3.84 (0.68)</td>
</tr>
</tbody>
</table>

* Parentheses for PLA microspheres diameter show standard deviation of 1000 spheres analyzed.
of NaCl or colloid-free influent to flush the system. Samples were collected every minute. The NaCl pulse was 15 min (6.3 pore volumes); the colloid pulse was 27 min (11.3 pore volumes). Cl⁻ was measured with a Buchler Digital Chlorimeter model 442–5000 (limit of detection (LOD) = 1% of the influent concentration (C<sub>i</sub>)). Concentrations of latex microspheres, bacteria, and PLA microspheres were measured with a fluorometer (LOD were, respectively, 2, 3, and 3% of C<sub>i</sub>). Special care was taken to preserve fluorophore integrity in PLA microspheres by minimizing exposure to light during experimentation. To remove fluorescent taggers released during PLA microsphere degradation, effluent samples were centrifuged (4000g, 4 °C, 2 min). 100 µL decanted from the supernatant, and resuspended in fresh autoclaved PBS. To account for colloids sorbed to sand grains, the sand with attached colloids was placed in 500 µL PBS and sonicated for 10 min, except bacteria which were sonicated 1 min, and the aqueous phase analyzed. Colloid retention within parts of the apparatus other than the porous media (e.g., tubing, syringe pump, etc.) was determined by running (in triplicate) empty flow-cell experiments for latex and PLA microspheres. We subtracted-out these apparatus-retracted colloids in subsequent analyses, so that mass balance and breakthrough curves represented only the colloids in the effluent or sand fractions. Thus C<sub>E</sub> was calculated as the percent recoverable in empty flow-cell experiments multiplied by the concentration placed in the syringe pump at the beginning of each experiment.

**Pore Scale Analysis.** Colloids on sand grain surfaces and in the aqueous phase were observed via microscopy (Leica TCS SP2 spectral confocal microscope, 10×, 0.4 UV objective), as described in Zevi et al. (<sup>9</sup>). Rhodamine B (0.0004%) was mixed with the colloid solution to visually distinguish water from air in the pores. The Leica system allowed for visualization of three separate channels: transmitted light showing grains, green fluorescence showing colloids (argon laser, excitation = 488 nm, emission = 543 nm) and red fluorescence showing water (HeNe laser excitation = 543 nm, emission = 638 nm), which could be superimposed onto a single image using the integrated Leica confocal software. Random locations in the column were observed to account for sand heterogeneity. At each location, a series of images (1 s time steps), collected over 60 s, were compiled using the Leica software; > 1000 images were captured for each colloid.

A threshold function in image J (<sup>25</sup>) was used on the green fluorescence image (showing colloid positions) based on intensity and converted to a binary image. The threshold function was used as a filter to eliminate colloids that were not in the focal plane but could still emit damped fluorescence. A threshold value was chosen for each series since fluorescent intensity could have some variation between pore locations due to the water depth above the focal plane and other condition variations. To quantify attached colloids on the grain, the in-focus portion of the grain surfaces, delineated by hand from the transmitted light image, was overlain with that from the green fluorescent images (colloids), to isolate colloids potentially attached to the grain surface and stored in a separate image. Image J was used to count the number of colloids that were at the same location between every pair of consecutive images; these colloids were considered attached, i.e., N<sub>a</sub>. A particle was considered attached when the colloid-pixels from two separate consecutive images overlapped by at least 2 pixels. A single colloid (area ~ 0.78 µm²) was approximately 3–5 pixels in images that were 512

![FIGURE 3. Four consecutive images of one of our pore-scale experiments in which we point out two colloids that are attached throughout the sequence (1) and (2) and another that attaches and then detaches during this period (3).](image)

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<table>
<thead>
<tr>
<th>colloid</th>
<th>attachment rate constant (h⁻¹)</th>
<th>detachment rate constant (h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>upper CI</td>
<td>lower CI</td>
</tr>
<tr>
<td>latex microspheres</td>
<td>616</td>
<td>703</td>
</tr>
<tr>
<td>bacteria (E. coli)</td>
<td>465</td>
<td>531</td>
</tr>
<tr>
<td>PLA microspheres</td>
<td>808</td>
<td>892</td>
</tr>
</tbody>
</table>

**TABLE 2. Attachment and Detachment Rate Constant Values Used to Model Column-Scale Transport. Upper and Lower 95% Confidence Interval (CI) Limits Were Used for Sensitivity Analysis on the Model**
× 256 pixels. The focal depth, \( d \), was 3–5 \( \mu \)m. Colloids in the pore space were similarly quantified by overlaying the pore area in the red fluorescence image with colloids from the green fluorescence image. Since the chamber was saturated, the grain surface in the transmitted light image overlapped with the pore area in the red fluorescent image. The pore area did not include grain surface areas.

The sequential composite images of attached colloids were compared to determine positive and negative changes in the number of attached colloids, \( \Delta N_g/\Delta t \) (eq 6) and, subsequently to calculate the attachment and detachment rate constants, separately (Figure 2). Equation 6 was rearranged to solve for the attachment and detachment rate constants assuming that \( k_d = 0 \) if \( \Delta N_g/\Delta t > 0 \) between a consecutive pair of images and visa versa for \( k_a \):

\[
  k_a = \frac{1}{N_p} \frac{\Delta N_g}{\Delta t} \quad (7)
\]

\[
  k_d = -\frac{1}{N_g} \frac{\Delta N_g}{\Delta t} \quad (8)
\]

This simplification results in underestimated rate constants when both attachment and detachment occur simultaneously within a 1 s time interval. All rate constants were calculated during Darcy-scale steady state conditions. Final rate constants were estimated by taking averages over 27, 29, and 39 image series for latex microspheres, bacteria and PLA microspheres, respectively (>50 images per series). Note, incidents when \( \Delta N_g = 0 \) were used in both attachment and detachment calculations. Figure 3 illustrates examples of attaching and detaching particles for a sequence of four consecutive images taken at 1 s intervals.

Results and Discussion

**Pore Scale Attachment and Detachment.** Average attachment rate constants (eq 7), ranged from 465 to 808 h\(^{-1}\) for bacteria and PLA microspheres, respectively (Table 2). The range of detachment rates (eq 8) was nearly twice as wide, from 302 to 1193 h\(^{-1}\) for PLA microspheres and bacteria, respectively. To evaluate whether a sufficient number of images was used to achieve representative rate constants, the constants were calculated 1000 times by randomly selecting 50% of the data. Rate constants calculated with randomly selected data were normally distributed, and fell within the 95% confidence limits (CI) of the respective data set for each colloid type (Table 2). Differences between the average rate constants and the confidence limits were between 9 and 14%.

**Darcy-Scale Breakthrough Curves.** The dispersion coefficient (\( D \)) was determined by fitting the one-dimensional convective-dispersion equation for a conservative, nonreactive substance (i.e., eq 1 with no storage term) to the Cl\(^-\) breakthrough data (Figure 4a): \( D = 54 \) cm\(^2\) h\(^{-1}\). Breakthrough curves for each type of colloid were modeled (eqs 1 and 2) using these values for \( D \) and \( v \) and attachment and detachment rate constants from the pore scale analysis (Figure 4). Although the attachment and detachment rate constants were determined from observations at a much smaller scale, the model worked well for all the microspheres, especially bacteria, which had standard error of the estimate (SEE) < 0.05 and \( R^2 = 0.99 \) (Figure 4). Indeed, although we can achieve better fits by optimizing the rate constants, we feel these uncalibrated results using independently determined parameters are more meaningful.

Although our results suggest that converting between pore-scale and Darcy-scale colloid behavior can work well
and can be physically consistent between the scales, we may have been fortunate in that $A_g/A_T$ in our images was similar to $(1 - n)$ at the Darcy scale. It is likely that we achieved good results in part because we used so many images to get average rate constants and, thus, probably inadvertently sampled a representative cross-section of the media heterogeneities.

One possible reason the model overestimated attachment in the breakthrough curve data during transient conditions for both latex and PLA microspheres (Figure 4) is that the microscopic images represented either too much poor space or too much sand grain relative to the bulk porosity, $n$. Another explanation for this discrepancy may be that our rate constants were determined from images of steady state flow and they may not capture the mechanisms during the rising and falling parts of the break-through curve. Both had a higher attachment than detachment rate constants and, as such, had deposition as the dominant mechanism, which may vary between transient and steady-state conditions. For example, the first colloids to attach may have been slower to do so due to differences in deposition between grains devoid of colloids and when other attached colloids are present (26). Indeed, as can be seen in Figure 4, the initial PLA microspheres broke-through earlier than predicted by the model, suggesting that the attachment rate constant was too high for this early period. Variable attachment/detachment rates have been seen by other researchers as well, e.g., bond aging (6, 27, 28). Another potential disconnect between our pore-scale rate constants and Darcy-scale model is unintentional bias in choosing observation sites with the microscope, i.e., colloid-free pores, if any existed, were never selected.

As mentioned earlier, previous colloid transport studies have often used optimized parameters to model rate constants. Bradford et al. (3) found modeled attachment and detachment rate constants to be $3.4 \times 10^5$ and $7.8 \times 10^4$ h$^{-1}$, respectively, for carboxylated latex microspheres in saturated sand. Although our study also found a higher attachment rate constant than detachment, the absolute differences between our constants and Bradford et al. (3) are huge. However, this could be due to differences in systems (ionic strengths, porous media, and flow rates). Tong et al. (5) reported an adhesion deficient bacterial ($Comamonas$ DA001) deposition and re-entrainment rate coefficients for distributed deposition as 2.86 and 1.57 h$^{-1}$, where ionic strengths (50 mM) and average linear velocity varied significantly from this study (33 cm h$^{-1}$ compared with 116 cm h$^{-1}$ in this study). Bradford et al. (4) reported modeled attachment and detachment rate constants for $Cryptosporidium$ oocysts in 1 mM NaBr of 6.6 and 0.78 h$^{-1}$, respectively. Although it is difficult to compare rates in this study with previous work because of experimental differences, studies that calibrate their system constants are always susceptible to the problem of getting the “right answer” for the “wrong reasons.” Indeed, in our study the shapes of latex microsphere and bacteria

![Figure 5](image-url)
breakthrough curves were similar but their rate constants were very different. Latex microspheres had a higher pore-scale attachment than detachment rate constant, whereas bacteria had a higher detachment rate constant. These mechanisms are different despite similar bulk breakthrough curve data and it is possible that rate constants calculated with the breakthrough curve best-fit could misrepresent transport mechanisms. For our experiments, best-fit attachment and detachment rate constants were as much as higher than observed at the pore scale. Whether optimized or based on pore-scale observations, our detachment rate constants were high compared to our attachment rate constants, which is why we observe very little “tailing” at the end of our experiments as one would expect to see in systems where larger colloids are irreversibly attached to the media. We suspect that much of our attachment was associated with a secondary energy minima, but additional experiments are needed to confirm this.

We tested how sensitive our model was to our attachment and detachment rate constants by rerunning the model using the pore-scale derived rate constants associated with the upper and lower 95% confidence intervals (Table 2) for all three colloid types (Figure 5). In all cases the model was insensitive to these changes in the rate constants with model variations much smaller than the variability in the experimental data. Thus, the disagreements between the model and measurements are either due to missing mechanisms in the model, e.g., straining or nonlinear attachment/detachment processes (e.g., ref 6), or problems in how we determined the rate constants.

There are a few yet unmentioned potential artifacts in the pore scale rate constant that may cause problems when applied to Darcy-scales. For example, fluorescent colloids just above or below the focal plane may still emit fluorescence in the images and, although a threshold value was used to “filter” the image during image analysis to minimize these effects, our fluorescent-based counts may introduce some error. Special care was taken to observe particle movement in each individual series before choosing a threshold value. Due to instrument limitations, we used a 1 s time duration to validate attachment of a single colloid. We found similar attachment and detachment values using time steps of both 1 and 5 s but we have no way of checking if a shorter time step would give different results. Unfortunately, it is unclear from the literature, how long a colloid needs to remain stationary on a grain surface to be considered “attached,” note the large range of published attachment coefficients noted earlier. It is also possible that our assumption that we can use the depth of the focal plane, d, used to transfer between 3-dimensional and 2-dimensional systems might introduce errors, especially at the edge of the pores where particle curvature into the image plane may introduce differences in areas at the top and bottom of the focal plane.

PLA microspheres had a significantly higher attachment rate constant than detachment and the attachment rate constant was also higher compared with other colloids. Breakthrough curve data for PLA microspheres was more attenuated, as compared to other colloids. PLA microspheres have been studied extensively for human therapeutic purposes, where engineering their surface properties has ranged from influencing hydrophobicity and size to surface charge. This information will be used to design future studies to better simulate natural colloids in the environment with PLA microspheres.

This study demonstrates that complexities in colloid transport are best addressed by research that considers both pore-scale phenomena and larger-scale transport responses. Indeed, this approach may uniquely provide additional insights needed to help answer a number of questions raised in this study, such as “how long should a colloid remain stationary before it is considered attached” or “can we link attachment times to specific attachment processes?”

Acknowledgments

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Supporting Information Available

Colloid preparation techniques and methods for determining various, relevant properties are described in detail. This materials is available free of charge via the Internet at http://pubs.acs.org.

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