CO$_2$-driven diffusiophoresis for removal of bacteria

Suin Shim$^1$ Sepideh Khodaparast$^2$, Ching-Yao Lai$^3$, Jing Yan$^4$, Jesse T. Ault$^5$, Bhargav Rallabandi$^6$, Orest Shardt$^7$, and Howard A. Stone$^1$

$^1$Department of Mechanical and Aerospace Engineering, Princeton University, Princeton, NJ 08544, USA
$^2$School of Mechanical Engineering, University of Leeds, Leeds LS2 9JT, UK
$^3$Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, USA
$^4$Department of Molecular, Cellular and Developmental Biology, Quantitative Biology Institute, Yale University, New Haven, CT, 06511, USA
$^5$School of Engineering, Brown University, Providence, Rhode Island 02912, USA
$^6$Department of Mechanical Engineering, University of California, Riverside, California 92521, USA
$^7$Bernal Institute and School of Engineering, University of Limerick, Castletroy, Limerick, V94 T9PX, Ireland

We investigate CO$_2$-driven diffusiophoresis of colloidal particles and bacterial cells in a Hele-Shaw geometry. Combining experiments and a model, we understand the characteristic length and time scales of CO$_2$-driven diffusiophoresis in relation to system dimensions and CO$_2$ diffusivity. Directional migration of wild-type V. cholerae and a mutant lacking flagella, as well as S. aureus and P. aeruginosa, near a dissolving CO$_2$ source shows that diffusiophoresis of bacteria is achieved independent of cell shape and Gram stain. Long-time experiments suggest possible applications for bacterial diffusiophoresis to cleaning systems or anti-biofouling surfaces.

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An aqueous suspension of charged particles in contact with a dissolving CO$_2$ source shows directional migration by diffusiophoresis [1, 2]. Here we use a Hele-Shaw geometry with either a CO$_2$ bubble [3, 5] or a CO$_2$-pressurized chamber to investigate the transport of polystyrene particles and bacterial cells. Combining experiments and model calculations, we understand the characteristic length and time scales of CO$_2$-driven diffusiophoresis in relation to system dimensions and CO$_2$ diffusivity. We then study migration of wild-type Vibrio cholerae and a mutant lacking flagella (ΔflaA) near a dissolving CO$_2$ source, showing that the directional motion is diffusiophoresis, not CO$_2$-driven chemotaxis. Also, we demonstrate diffusiophoresis of Staphylococcus aureus [6] and Pseudomonas aeruginosa [7], showing that diffusiophoresis driven by CO$_2$ dissolution occurs for both Gram-positive and Gram-negative bacteria, independent of shape. We further demonstrate that diffusiophoretic removal of S. aureus reduces cell adhesion to a surface, and that the removal of P. aeruginosa lasts ≥11 hr after CO$_2$ is turned off. CO$_2$-driven diffusiophoresis can prevent surface contamination or infection by reducing the population of cells approaching an interface, and the mechanism can be applied to liquid cleaning systems and anti-biofouling surfaces.

When an aqueous suspension of charged colloidal particles is exposed to dissolving CO$_2$, positively (negatively) charged particles migrate toward (away from) the CO$_2$ source by diffusiophoresis [1, 2]; the fast diffusing H$^+$ relative to HCO$_3^-$ from the dissolution of H$_2$CO$_3$ drives the transport. We investigate the phenomenon using a Hele-Shaw geometry (Fig. 1(a) a circular cell with radius $b = 11$ mm and height $h = 500$ μm). Here, diffusiophoresis near a CO$_2$ source is documented experimentally and calculated (Supplementary Information; SI) for two configurations – a dissolving CO$_2$ bubble (Fig. 1(a)); we call this system HS-B) and a CO$_2$-pressurized chamber (Fig. 1(b)); HS-PC) – to examine both moving and fixed boundaries.

In HS-B, a CO$_2$ bubble with radius $a(t)$ and an initial radius $a_0$ dissolves at a typical speed $da/dt \approx D_0/\sigma_0 \approx O(0.1-1) \mu m/s$ until the gas exchange reaches steady state [1, 3–5], where $D_1$ is the diffusivity of CO$_2$ in water. A bubble reaches its steady state within $\tau = a_0^2/D_1 \approx 1$ (SI). The typical diffusiophoretic velocity of nearby suspended particles $u_p = G_p \sqrt{\Gamma_c c_i}$ scales as $u_p \approx \Gamma_p a_0 \approx O(0.1-1) \mu m/s$, where $G_p$ and $c_i$ are the diffusiophoretic mobility of particles and concentration of ions, respectively (SI, video 1). The relative motion of particles and the interfaces creates a charge-dependent particle distribution for both HS-B and HS-PC (Fig. 1(c-f)).

Locally, in the vicinity of the interface, amine-modified polystyrene (a-PS, positively charged, diameter = 1 μm) particles accumulate and form a high particle-density region, whereas polystyrene (PS, negatively charged, diameter = 1 μm) particles create an exclusion zone (EZ; Fig. 1(c-f)), where the particle concentration is small. Growth of the local EZ in HS-PC (SI Fig. S2) is proportional to $\sqrt{t}$, similar to EZ formation near an ion-exchange membrane [8].

Particle accumulation and exclusion also occur on the length scale $\ell \approx a_0$ in both systems (Videos 2,3). In the model we define the boundaries of macroscopic accumulation and exclusion as the radial distance where the nondimensional particle concentration $\bar{n} = n/n_0 = 1$ ($n(r,t)$ is the particle concentration and $n_0 = n(r,0)$;
FIG. 1. CO$_2$-driven diffusiophoresis of colloidal particles. (a-h) Schematics of experimental setup for (a) HS-B and (b) HS-PC. See SI for details. (c,d) Charged particles near a dissolving CO$_2$ bubble (HS-B). Distribution of (c) amine-modified polystyrene (a-PS) particles and (d) polystyrene (PS) particles show, respectively, local accumulation and exclusion of charged particles by diffusiophoresis. Bright dots indicate particles. (e,f) Charged particles near the CO$_2$ source in HS-PC. Distribution of (e) a-PS and (f) PS particles near the fixed CO$_2$ source show local accumulation and exclusion. (g,h) Comparison between experimental measurements and model calculations of the macroscopic growth of the accumulation and exclusion zones. (g) Measured and calculated values of $\hat{r}(\bar{n} = 1)$ are plotted versus $\tau$ for HS-B. (h) Measured and calculated values of $\hat{r}(\bar{n} = 1)$ are plotted versus $\tau$ for HS-PC. (g,h) No fitting parameter is used. (c-f) Scale bars are 500 $\mu$m.

SI). The nondimensional radial positions are defined as $\bar{r} = r/a_0$ for HS-B, and $\hat{r} = \frac{r - a}{b - a}$ for HS-PC. Such boundaries are determined analogously in the experiments (SI) and plotted versus $\tau$ in Fig. 1(g,h). For HS-B, the boundaries grow faster in experiments due to the initial rapid generation of the bubble, which is not included in the model. Bubble generation introduces fast interface growth, which enhances CO$_2$ dissolution at the early times and causes faster diffusiophoresis. The particle dynamics show better agreement in HS-PC. Without the initial growth in the measured boundaries in HS-B, we obtain similar trends of the particle dynamics between HS-B and HS-PC (SI).

The macroscopic boundaries increase up to almost half of the radius of the Hele-Shaw cell ($\approx 0.5b$) within $\tau = 0.2$ in HS-PC. In HS-B, as noted from the time evolution of the radius (SI), there is a velocity contribution from the shrinking bubble $(da/dt)$ that affects the particle distribution, and this effect lasts up to $\tau \approx 1$.

Our understanding of the typical length and time scales of CO$_2$-driven diffusiophoresis in a Hele-Shaw cell motivated us to extend our investigations to a broader range of particles. Past studies have reported on the use of diffusiophoresis to achieve migration of living cells [9, 10]. For example, the goals of particle manipulation can be to clean a region of liquid, achieve antifouling surfaces, or prevent infection in biological systems. Two previous studies report EZ formation in bacterial suspensions in contact with an ion-exchange membrane (Nafion) [11, 12] and discuss possible cleaning applications.

As an initial step for demonstrating and investigating diffusiophoresis of bacterial cells by CO$_2$ dissolution, we chose two types of $V.\$ cholerae$ cells – wild-type (WT) and a mutant lacking flagella ($\Delta$flaA), both of which are tagged by mKO (monomeric Kusabira Orange), a bright fluorescent protein [13]. We first confirm the diffusiophoretic contribution to the cell migration in the presence of a dissolving CO$_2$ source in a Hele-Shaw geometry. Then, using PIV, we measure the velocities of the bacterial cells that move along the ion concentration gradient.

$V.\$ cholerae$ is Gram-negative, comma-shaped (length $\approx 2-3 \mu$m, diameter $\approx 1 \mu$m), and single flagellated. The net surface charge of $V.\$ cholerae$ (as well as other bacteria) is negative [14, 15], so the cells are expected to migrate away from a CO$_2$ source by diffusiophoresis (Video 4). We prepared a bacterial solution by diluting the growth suspension (see SI for Methods) to 10% M9 minimal salt solution. No nutrient is provided so no growth and division occur on the time scale of the experiment. Using low salt concentration helps to exclude effects of coupled ion fluxes on the diffusiophoresis of bacteria [16]. Similar to the particle experiments, we fill the Hele-Shaw cell with bacterial suspension, and introduce either a CO$_2$ bubble or pressurize CO$_2$ in the inner chamber. Fluorescence intensities near the CO$_2$ source for both HS-B and HS-PC systems are measured (Fig. S10), and the intensity change shows that the cell number near the dissolving CO$_2$ source decreased significantly over time.

Particle image velocimetry (PIV) near the fixed boundary measures the diffusiophoretic velocity of the cells by a dissolving CO$_2$ source. We plotted the velocity vectors
aligned velocity vectors in the radially outward direction. The directional migration of cells is described by $\tau$ versus position in Fig. 2(a,b), where the origin of the plotted versus $r^2$.\(\text{FIG. 2. Velocity measurements for CO}_2\)-driven diffusiophoresis of V. cholerae. (a,b) PIV for V. cholerae cells in the HS-PC experiments. Velocity vectors plotted versus position ($r - a, z$). Motion of (a) wild-type and (b) $\Delta$flaA cells at $t \approx 10$ minutes. The directional migration of cells is described by aligned velocity vectors in the radially outward direction. (c) Nondimensional $z$-averaged velocities obtained from (a,b) and control experiments without $\text{CO}_2$ at $\tau = 0.15$ ($\approx 10$ minutes) plotted versus $r - a$. (d) Nondimensional $z$-averaged velocities of PS particles, WT and $\Delta$flaA cells obtained at $\tau = 0.15$ plotted versus $r - a$.

versus position in Fig. 2(a,b), where the origin of the $z$-axis is at the bottom left corner. After the $\text{CO}_2$ valve is opened at $\tau = 0$, both strains of V. cholerae migrate radially outward (Fig. 2(a,b)). The radial alignment of the velocity vectors confirms that both motile and immotile V. cholerae cells move along the CO$_2$-generated ion concentration gradient. In Fig. 2(c), nondimensional $z$-averaged velocities ($u_{\text{cell}} = u_{\text{cell}}/(D_1/a)$; $u_{\text{cell}}$ is the $z$-average of measured velocity) of the cells at $\tau = 0.15$ with and without dissolving $\text{CO}_2$ are plotted. Our observation that both motile and immotile cells exhibit directional migration with similar velocities shows that the motion is not a chemotactic effect. We also compare the typical velocity scales of the cells and the PS particles in Fig. 2(d). The diffusiophoretic velocity of the bacterial cells is smaller than that of the PS particles, and as a first rationalization, this is due to the smaller diffusiophoretic mobility of the cells. Our comparison suggests that the V. cholerae cells have three to four times smaller mobility compared to the PS particles, since the diffusiophoretic velocity scales as $u_p \approx \Gamma_p/a$. To highlight the generality of the phenomenon, two more bacteria were examined – S. aureus (mKO labeled, Gram-positive, spherical and immotile) [7] and P. aerugino-
and observe that, by CO₂ diffusiophoresis, bacterial cells move away from the inner wall, whereas without any CO₂ source, the cells concentrate near both inner and outer PDMS walls where there is an air source. The CO₂ valve was open only for 1 hr, but the result of diffusiophoresis lasted longer than 12 hours (Fig. 3f)). The distribution of the cells at t = 12 hr are presented in the SI.

Finally, we discuss the diffusiophoresis of *motile* bacteria since it is not identical to that of polystyrene particles or immotile cells. Both *V. cholerae* and *P. aeruginosa* are single flagellated organisms and exhibit run-reverse patterns [24]. The effective diffusivity of motile bacteria with typical translational speed $v_t$ and reverse time $t_r$ can be estimated as $D_{\text{eff}} \approx v_t^2 t_r \approx O(100) \, \mu m^2/s$ (SI). It is observed (Video 5) that the flow of cells under ion concentration gradient is a slow advection with an estimated Pécel number $Pe = \frac{u_p \ell_{cell}}{D_{\text{eff}}} \approx 10^{-3}-10^{-2}$. Cells are observed to swim randomly with their characteristic velocity $\approx 30-50 \, \mu m$ (SI), with a slow drift (radially outward) due to the diffusiophoretic contribution (Video 5).

In this paper, we present proof of diffusiophoretic migration of different types of bacteria under a concentration gradient of CO₂, and discuss possible applications of CO₂-driven diffusiophoresis to prevent contamination. For example, delaying biofilm formation can improve the anti-biofouling properties of surfaces. Currently we are working to realize the mechanism at various salt concentrations to broaden the understanding to physiological or higher salinity conditions. Moreover, understanding the characteristic scales and flow structure near the CO₂ source is crucial for the next steps of CO₂-driven diffusiophoresis for mitigating bacterial growth on, or bacterial removal from, surfaces.

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CONTRIBUTIONS

S.S. and H.A.S. conceived the project. S.S. designed and performed all experiments. S.K. conducted PIV. S.S., C.Y.L., J.T.A. conducted numerical calculations. J.Y. constructed the V. cholerae strains. S.S., B.R., O.S., and H.A.S. set up the theoretical model. All authors contributed to data analysis and writing the paper.

1 shim@princeton.edu
2 hastone@princeton.edu

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