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Cell and Particle Interactions and Aggregation
During Electrophoretic Motion

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1. Objectives

The objectives of this research were (i) to perform experiments for observing and quantifying electrophoretic aggregation, (ii) to develop a theoretical description to appropriately analyze and compare with the experimental results, (iii) to study the combined effects of electrophoretic and gravitational aggregation of large particles, and the combined effects of electrophoretic and Brownian aggregation of small particles, and (iv) to perform a preliminary design of a potential future flight experiment involving electrophoretic aggregation. Electrophoresis refers to the motion of charged particles, droplets or molecules in response to an applied electric field. Electrophoresis is commonly used for analysis and separation of biological particles or molecules. When particles have different surface charge densities or potentials, they will migrate at different velocities in an electric field. This differential migration leads to the possibility that they will collide and aggregate, thereby preventing separation.

2. Accomplishments

2.1 Microphysical theory of electrophoretic aggregation

Our previous work on pairwise aggregation of particles with different zeta potentials in an electric field (Nichols et al., 1995) was extended to combined gravity and electrophoretic motion (important for large particles), combined Brownian and electrophoretic motion (important for small particles), and to additional parameter values which include those of the suspensions used in the experiments. The combined gravity and electrophoretic motion is of particular interest, both because gravity sedimentation is expected to play a contributing role in the ground-based experiments and because it may be possible to suppress aggregation by appropriate manipulation of the relative orientation and magnitude of the gravity vector and the electric field.

A theoretical study of pairwise aggregation of particles undergoing combined electrophoretic and gravitational motion was completed (Wang et al., 1997). Depending on its relative strength and orientation, the presence of an electric field may enhance or reduce the gravitational aggregation rate. For antiparallel alignment of gravitational and electrophoretic motion, with the former dominant, a “collision-forbidden” region of parameter space is predicted due to the stronger hydrodynamic interactions of two particles for gravitational motion than for electrophoretic motion. This region is evident in Figure 1, which shows the predicted collision frequency (number of doublets formed per total number of particles per unit time) for a model system. Figures 2 and 3 show how one particle moves outward around the other for antiparallel alignment with gravitational motion dominant, while one particle moves inward toward the other for antiparallel alignment with electrophoretic motion dominant, respectively.

The mobility functions for two interacting spheres of different size and surface potential undergoing electrophoresis with arbitrary separation and orientation of the line of centers relative to the electric field were determined (Zeng et al., 1999a). These results are used for calculations of particle trajectories and aggregation rates. Previous solutions for these functions have not yielded accurate results for all size ratios or for when the two particles come very close together. The new method of calculating mobility functions is highly accurate and efficient. Figure 4 shows the dimensionless collision efficiency (defined as the collision rate with hydrodynamic and interparticle interactions to the rectilinear collision rate without interactions) for two unequal spheres versus the dimensionless driving force (ratio of electrophoretic forces and van der Waals attractions). For small driving forces, the particles are captured in a weak secondary minimum, whereas they aggregate in the strong primary minimum for large driving forces. At intermediate driving forces, there is a stable region for which the electric field is strong enough to pull the particles out of the secondary minimum but not overcome the electrostatic-repulsion barrier and push them into the primary minimum.
The theory of colloidal particles aggregation due to the combined effects of electrophoresis and Brownian motion has also been completed (Wilson et al., 2000). When electrophoretic forces are too weak to push the particles over the repulsive barrier so that they aggregate, but are sufficiently strong to push the particles a significant way up the barrier, then a small amount of random Brownian motion is able to cause the particles to cross the barrier and aggregate at a rate that is greatly enhanced over the rate from either individual mechanism. Figure 5 shows the collision efficiency versus electric field strength for different pairs of particles. The enhancement in the collision rate due to the synergistic effect of electrophoresis and Brownian motion is an order of magnitude, or more, at intermediate field strengths!

2.2 Ground-based experiments on electrophoretic motion and aggregation

We performed zone electrophoresis in which a band of higher-mobility particles passes through a band of lower-mobility particles so that a third band, composed of heteroaggregates with intermediate mobility, is formed. Gravity sedimentation and buoyancy effects are partially suppressed using a density gradient in a vertical configuration. The chamber is designed so that samples may be withdrawn and analyzed microscopically. The density-gradient zone electrophoresis chamber uses a combination of a sucrose density gradient and a cooling jacket to stabilize against free convection due to electroviscous heating.

Experiments were performed in which a band of faster-moving particles passes through a band of slower-moving particles, resulting in aggregate formation (Zeng, 1998; Davis et al., 1998). In the initial experiments, polystyrene latex particles of 3.5 μm diameter and two different surface charges (due to different surface coverages of sulfate groups) were used. Aggregation was observed and quantified, but it could not be determined if homoaggregates or heteroaggregates formed, since the particles are physically indistinguishable. In more recent experiments, polystyrene latex particles of 2.3 μm diameter and 3.5 μm were used. As shown in Figure 6, heteroaggregates were formed when a band of faster-moving particles passed through a band of slower-moving particles. As expected, increasing the particle concentrations gave a higher relative amount of aggregation (Figure 7). However, a major difficulty is that the two original bands do not completely separate, leaving open the possibility that aggregates form during sampling from the region of band overlap. Zeng (1998) has verified that aggregation during sampling does occur and can obscure the results.

One reason that the bands do not completely separate is that they experience considerable spreading as they travel up the column. Figure 8 shows the spreading of a single band which initially contained 0.01% particles by volume. A small amount of apparent spreading can be attributed to loading and unloading samples, but significant spreading also occurred during electrophoresis. The spreading is not reversible (the band did not become narrow again upon reversal of the electric field), and so heterogeneity in zeta potential does not appear to be the main reason for band spreading. Moreover, the rate of band migration unexpectedly decreased as the band ascended the column. Figure 9 provides further data on the decrease in band velocity with time. We observed large pH changes on the column (presumably due to electrochemical reactions at the electrodes) during electrophoresis, and it is expected that these changes affected the particle mobilities and caused the observed variations in band velocity and spreading (Zeng et al., 1999b).

2.3 Macrophysical theory of electrophoretic aggregation

To properly interpret the experimental results, a macroscopic theory capable of predicting the number density of aggregates versus position and time as one band passes through the other was developed using an inhomogeneous population dynamics model together with the collision kernels.
for pairwise aggregation predicted by the microphysical theory. An interesting extension is the analysis of the formation of multiplets comprised of many primary particles with two (or more) different zeta potentials. Unlike in gravity sedimentation, for which the aggregate velocity increases monotonically with aggregate size, the electrophoretic aggregate velocity is nearly size-independent and so relative motion and further aggregation may be suppressed once aggregates grow beyond some small size.

Typical results from the macrophysical theory for zone electrophoresis with two initial bands of single particles which pass through each other to form a third band of dimers are shown in Figure 10. The theory gives results which are qualitatively similar to the experimental observations. However, the spreading of the bands in the experiments is greater than predicted by the current theory, as discussed previously. Additional work has been performed on the interaction of multiple particles, as will be needed to follow the growth of aggregates beyond doublets.

3. Significance

Electrophoresis is widely used on the analytical scale (and sometimes on the preparative scale) for the separation of biological molecules such as proteins and nucleic acids. To improve the separation and reduce convection due to electrical heating, such separations are usually done in a gel matrix or a capillary tube. A suspension of particles or cells would clog a gel matrix or very fine capillary, however, and so free electrophoresis of the suspension is performed. In this case, the separation efficiency may be affected by three factors: (i) free convection or mixing due to thermal gradients, (ii) particle sedimentation, and (iii) particle aggregation. Our research focused on particle aggregation due to electrophoretic motion with and without gravity sedimentation. The ground-based experiments were designed to minimize free convection, by employing a density gradient, but it is anticipated that a low-gravity environment would be needed to study electrophoretic aggregation in the absence of sedimentation and convection. However, because of the observed spreading and decrease in velocity as the bands traveled up the long column, the initial design does not appear to be the best choice for studying heteroaggregation even in a microgravity environment. Instead, multiple stages, each of which is much shorter than the current column, are expected to more effectively yield the desired separation and aggregation.

In addition to having general scientific and engineering significance, our fundamental study of particle aggregation in electric fields is expected to have practical application to electrically-controlled cell flocculation for cell separation and recycle in space-based bioreactors, where gravity cannot be employed. Similarly, an extension of the proposed study to drop interactions and coalescence would provide an understanding of electrically-driven demixing of two liquid phases, such as those encountered in biphasic aqueous extraction of biological cells and molecules under reduced gravity when buoyancy-driven demixing is weak.

4. Publications/Presentations


Zeng, S., Todd, P. W., and Davis, R. H., “Particle Aggregation During Electrophoretic Motion”, Annual AIChE Meeting, Chicago, IL, November 1996.


5. **Honors/Awards/Citations**

Professor Davis was selected by students in the Department of Chemical Engineering at the University of Colorado to receive the 1995-96 Outstanding Graduate Teaching Award.

Shulin Zeng won a Third-Place Outstanding Student Poster Award for his presentation at the 1996 Annual AIChE Meeting.

Professor Davis was appointed as Patten Professor of Chemical Engineering, effective July 1997.

Tyson Ingels received an Undergraduate Research Opportunities Program award from the University of Colorado for his participation in the research.

Professor Davis received a Faculty Fellowship for sabbatical at the University of California, Santa Barbara, 1997-98.

Professor Davis won the 1998-99 Max S. Peters Outstanding Service Award for the College of Engineering and Applied Science at the University of Colorado.

Professor Davis won the 1999-2000 Charles Hutchinson Memorial Teaching Award from the College of Engineering and Applied Science at the University of Colorado.

Professor Davis won the Boulder Faculty Assembly Excellence in Research and Creative Work Award from the University of Colorado.

6. **Students Supported**

The grant has supported the work of one PhD student (Shulin Zeng), one MS student (Lorraine Pietraszewski), and two Research Associates (Alexander Zinchenko and Helen Wilson). Three undergraduates (Vannessa Aponte-Irizarry, Matt Goebel, and Tyson Ingels) also assisted with the experimental work.
Figure 1 - The aggregation frequency as a function of the radius of the larger particle for a model system with gravitational motion alone, electrophoretic motion alone, parallel combined motion, and antiparallel combined motion. For antiparallel combined motion, the collision forbidden region is evident for $2 \mu m < a_1 < 6 \mu m$.

Figure 2 - The grazing relative trajectory for gravitational motion ($N_E = 0$) and relative trajectories with the same impact parameter for antiparallel combined motion when electrophoresis is weak ($N_E$ is the ratio of electrophoretic motion to gravitational motion).

Figure 3 - The relative trajectories for electrophoretic (dashed curve), gravitational (solid curve), and antiparallel combined motion (dotted curve) when electrophoresis is stronger than gravity.
Figure 4 – Electrophoretic collision efficiency versus dimensionless driving force, $N_A$, for two particles with a zeta potential ratio of $\beta = 0.5$. The lines are calculated using the mobility functions from Zeng et al. (1999a) and are for size ratio $\lambda = 1, 2, 3, 4, 5, 6, 7, 8, 9,$ and $10$ from top to bottom at large and small $N_A$. The symbols are calculated using the mobility functions from previous works and are for $\lambda = 1$ (O), $2$ (O), and $5$ (Δ).

$Q_E$ for the mixed pair; $a_1 = 1 \, \mu m$, $a_2 = 3 \, \mu m$.

Figure 5 – Collision efficiencies for different pairs of particles, plotted against the electric field $E$. On the top axis are shown the values of a dimensionless driving force representing the ratio of electrophoretic forces to van der Waals forces for the small-large interactions. The lines represent, reading from top to bottom at the left-hand side, small-small, small-large, and large-large collisions, respectively. The small and large particle radii are 1 and 3 $\mu$m, respectively, and each interaction is between a pair of particles having charges 10 and 20 mV. The collision efficiencies which would result from Brownian motion alone are given by the dashed lines, and those for electrophoretic motion alone by the solid curves. The points (×) show the actual collision efficiency.
Figure 6 - Particle number density distribution in the column after electrophoresis of 0.01% 2.3 μm particles (dotted line) and 0.03% 3.5 μm particles (solid line) to form heteroaggregates (dashed line).

Figure 7 - Particle number density distribution in the column after electrophoresis of 0.02% 2.3 μm particles (dotted line) and 0.05% 3.5 μm particles (solid line) to form heteroaggregates (dashed line).

Figure 8 - Number-density distributions of particles after electrophoresis for 0, 30, 60, and 90 min (solid lines). The dotted line is the calculated initial distribution of the band, and the dashed line is the particle distribution after 60 min of upward electrophoresis followed by 90 min of downward electrophoresis. The inset is a schematic representation of the density-gradient zone electrophoresis apparatus: (A) 15% sucrose floor solution, (B) 12 to 0% sucrose density-gradient solution, (C) 0% sucrose ceiling solution, (D) three-way stopcock, (E) electrode with top solution, (F) saturated KCl solution, (G) 15% polyacrylamide gel plug, (H) cooling jacket (cooling finger in center of column not shown), (I) particle band, which is also shown in expanded form (not to scale) for negatively charged particles surrounded by counterions.
Figure 9 – Net distances traveled by bands versus running time for electrophoresis at different particle concentrations. The symbols are averages of 3-4 repeats, and the error bars on selected points represent typical 90% confidence intervals. The inset gives the distance traveled by a band of 0.03% particles by volume subject to 60 min each of forward, zero, reverse, zero, and forward electric fields.

Figure 10 – Simulation results for density gradient zone electrophoresis of faster (solid lines) and slower (dotted lines) particles which form heteroaggregates (dashed lines).