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## ELECTROPHORETIC INTERACTIONS AND AGGREGATION OF COLLOIDAL BIOLOGICAL PARTICLES

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### ABSTRACT

The separation of cells or particles from solution has traditionally been accomplished with centrifuges or by sedimentation; however, many particles have specific densities close to unity, making buoyancy-driven motion slow or negligible, but most cells and particles carry surface charges, making them ideal for electrophoretic separation. Both buoyancy-driven and electrophoretic separation may be influenced by hydrodynamic interactions and aggregation of neighboring particles. Aggregation by electrophoresis was analyzed for two non-Brownian particles with different zeta potentials and thin double layers migrating through a viscous fluid. The results indicate that the initial rate of electrophoretically-driven aggregation may exceed that of buoyancy-driven aggregation, even under conditions in which buoyancy-driven relative motion of noninteracting particles is dominant.

### INTRODUCTION

Electrophoresis is a common analytical technique for separating charged biological particles and macromolecules. In an electric field, suspended particles will migrate at a velocity proportional to their surface charge and the applied field strength. Smoluchowski [1] showed that, for thin double layers, the electrophoretic velocity of an isolated particle is:

$$v^{E,0} = \left( \frac{\epsilon \zeta}{\mu} \right) E_{\infty} \quad (1)$$

where  $\epsilon$  is the dielectric constant,  $\zeta$  is the zeta potential of the charged particle surface,  $E_{\infty}$  is the applied electric field,  $\mu$  is the viscosity of the fluid, and the group in parenthesis is the electrophoretic mobility,  $\mu_e$ , a physical constant of a given particle. Morrison [2] later showed that the electrophoretic mobility was independent of the size and shape of the particle.

In a heterogeneous suspension, particles with different surface charges will have different electrophoretic velocities, leading to the possibility that they will collide and aggregate [3]. Such aggregation inhibits the ability for different subpopulations of cells or other biological particles to be separated and is the subject of this article. Particle aggregation has been investigated theoretically for buoyancy-driven motion by Davis [4] and Melik and Fogler [5] who used a trajectory analysis to determine the aggregation rates. A similar analysis for electrophoretic particle aggregation is performed in this work and compared with the results for gravity-induced aggregation.

### THEORETICAL DEVELOPMENT

We restrict our attention to suspensions which are dilute, so that only two particles interact at one time. The particles are assumed to have negligible Brownian motion and the surrounding fluid is assumed to have negligible inertia. The zeta potentials are of moderate strength or less, and the electrical double layers are thin ( $\kappa a \gg 1$ ). These conditions are typically met for biological cells and other charged particles of 1-50  $\mu\text{m}$  radius.

To study coagulation and stability of dilute suspensions of spherical particles, a quantitative description of the relative motion between two spheres is needed. When the particles are far apart, this relative motion

is simply the difference between the separate velocities, given by:  $\mathbf{V}_{12}^o = \mathbf{V}_1^o - \mathbf{V}_2^o$ . For electrophoresis,  $\mathbf{V}_{12}^o = (1 - \beta) \mathbf{V}_1^{E,o}$ , where  $\mathbf{V}^{E,o}$  is defined by eq. (1),  $\beta$  is the ratio of zeta potentials,  $\zeta_2/\zeta_1$ . For gravity sedimentation,  $\mathbf{V}_{12}^o = (1 - \lambda^2\gamma) \mathbf{V}_1^{G,o}$ , where  $\mathbf{V}^{G,o}$  is the Stokes sedimentation velocity [4]:

$$\mathbf{V}^{G,o} = \frac{2\Delta\rho a^2 \mathbf{g}}{9\mu} \quad , \quad (2)$$

$\Delta\rho$  is the density difference between the particle and the fluid,  $a$  is the particle radius,  $\mathbf{g}$  is the gravitational acceleration,  $\lambda$  is the particle size ratio,  $a_2/a_1$ , and  $\gamma$  is the reduced density ratio,  $\Delta\rho_2/\Delta\rho_1$ .

As two particles approach each other, hydrodynamic interactions significantly affect the relative velocity, and this can be expressed through the use of mobility functions. Using the coordinate system shown in Fig. 1, we write [6]:

$$U_{12,r} = -L(r) \cos \theta - \frac{G(r)}{N_A} (f_A - N_R f_R) \quad , \quad (3)$$

$$U_{12,\theta} = M(r) \sin \theta \quad , \quad (4)$$

where  $U_{12,r}$  and  $U_{12,\theta}$  are the components of the relative velocity directed parallel and perpendicular to the line of centers, respectively; the velocities are made dimensionless by  $V_{12}^o$ , and  $r$  is the distance between the centers of the two particles made dimensionless by the average particle radius. The mobility functions  $L(r)$  and  $M(r)$  describe relative motion due to the driving force. The mobility function  $G(r)$  describes relative motion due to interparticle forces, which are summarized later in this section. When the dimensionless interparticle separation distance,  $\delta = r - 2$ , is large compared to unity,  $L(r)$ ,  $M(r)$ , and  $G(r)$ , approach unity, but, at near contact,  $L(r)$  and  $G(r)$  approach zero, and  $M(r)$  tends to a finite limit.

Figure 2 shows  $L(r)$  for electrophoretic and buoyancy motion. For electrophoresis,  $L(r)$  was obtained from the method of reflections [7] at large separations, a boundary collocation solution [8] at moderate separations, and a lubrication solution [9] at small separations. For buoyancy,  $L(r)$  is taken from the solutions summarized by Jeffery and Onishi [10]. The mobility functions for electrophoresis approach unity much more rapidly (with a leading-order correction proportional to  $1/r^3$ ) than do those for buoyancy (which have a leading-order correction proportional to  $1/r$ ) as the interparticle separation is increased; this is because electrophoresis is a force-free motion.

When the interparticle separation distance is small, short-range interparticle forces will affect the relative particle motion. Electrostatic repulsion due to overlapping double layers can keep particles from aggregating, if strong enough. However, in most cases, van der Waals attractive forces will cause aggregation to occur. It will be assumed that these colloidal forces act only along the line of centers. The driving force for aggregation made dimensionless by the van der Waals force is described by the dimensionless parameter

$$N_A = \frac{6\pi\mu V_{12}^o a^2}{A} \quad , \quad (5)$$

where  $A$  is the Hamaker constant and  $a$  is the average particle radius. A dimensionless function describing the attractive van der Waals forces, taken from Gregory [11], is

$$f_A = \left( \frac{1}{6\delta^2} \right) \left( \frac{\nu}{5.32\delta + \nu} \right) \quad , \quad (6)$$

where  $\delta = h/a$  is the dimensionless particle separation distance, and  $\nu = \lambda_L/a$  is the dimensionless retardation parameter, with  $\lambda_L \approx 0.1 \mu\text{m}$  being the London retardation wavelength. This expression is valid for  $\delta \ll 1$ , and, since van der Waals attractions are negligible at larger separations, eq. (6) will be used throughout. The dimensionless parameter describing the strength of the electrostatic repulsion relative to the van der Waals attraction is

$$N_R = \frac{\epsilon\zeta_1^2 a}{A} \quad . \quad (7)$$

The dimensionless function describing the electrostatic repulsion force due to overlapping double layers, taken from Hogg *et al.* [12], is

$$f_R = \kappa a \left( \frac{\beta e^{-\kappa a \delta} - \left(\frac{1+\beta^2}{2}\right) e^{-2\kappa a \delta}}{1 - e^{-2\kappa a \delta}} \right) \quad (8)$$

This expression for the double layer repulsion is valid for  $\kappa a > 10$ ,  $\zeta_1 < 60$  mV, and constant surface potential.

## RESULTS

Colloidal suspensions tend to form aggregates of particles due to van der Waals attraction, but electrostatic repulsion between two particles can lead to a stable suspension. To characterize the stability of the suspension, we examine the relative motion along the line of centers using eq. (3). A stable suspension is one in which the relative velocity along the line of centers,  $U_{12,r}$ , becomes zero at finite separations for all values of  $-1 \leq \cos \theta \leq 1$ . Moreover, the location at which  $U_{12,r} = 0$  is stable only if  $dU_{12,r}/dr < 0$ , indicating that a pair of particles will move apart if they become slightly closer than the stable separation distance.

Melik and Fogler [5] have previously studied the stability of particles undergoing gravity sedimentation. We show here how their results can be extended to electrophoretic motion. In particular, if eq. (3) is written for gravity motion and electrophoretic motion, and set to zero relative velocity for each case, we have

$$N_A^E = \frac{L^G}{L^E} N_A^G \quad (9)$$

along the neutral stability curves. Since  $L^G/L^E \ll 1$  at small separations (see Fig. 2), this implies that electrophoretic motion becomes unstable at larger dimensionless driving forces than does buoyancy motion (Fig. 3). Moreover, since interparticle forces act at distances where near-contact hydrodynamics apply,  $L^E/L^G$  is independent of the small separation distance because  $L(r)$  is proportional to the gap between the particles for both types of motion [9].

Loewenberg and Davis [9] have analyzed electrophoretic near contact motion and compared the results with buoyancy-driven near-contact motion. They find for small size ratios,  $\lambda < 0.2$ ,  $L^E/L^G$  can be approximated by

$$\frac{L^E}{L^G} = \frac{1.89}{\lambda(1 - 0.206\gamma)} \quad (10)$$

Then at small size ratios,  $L^E/L^G$  is proportional to  $\lambda^{-1}$ , which implies that electrophoresis is able to force particles together much easier than gravity is able to. In fact,  $L^E/L^G > 8$  for all size ratios at  $\gamma = 1$ ; thus, electrophoresis is much less stable than buoyancy. Loewenberg and Davis [9] have postulated that the greater tendency for particles to aggregate when undergoing electrophoresis is due to electroosmotic convection which draws fluid out of the gap between two approaching particles. In contrast, the fluid must be squeezed out of the gap between two approaching particles in buoyancy-driven motion, which causes greater resistance to close approach.

## AGGREGATION RATE RESULTS

### Trajectory Analysis

The pairwise aggregation rate is equal to the flux of particle pairs through an upstream collision cross-section [4]:

$$J_{12} = n_1 n_2 V_{12}^0 \pi \sigma_c^2, \quad (11)$$

where  $n_i$  is the number density of particles of type  $i$  and  $\sigma_c$  is the critical impact parameter at upstream infinity defined in Fig. 4 such that particle capture occurs only for  $\sigma \leq \sigma_c$ . The collision efficiency is defined

as the actual collision rate given by eq. (11) divided by that in the absence of particle-particle interactions ( $\sigma_c = a_1 + a_2$ ):

$$E_{12} \equiv \left( \frac{\sigma_c}{a_2 + a_1} \right)^2 \quad (12)$$

The critical impact parameter and the collision efficiency were determined from a trajectory analysis [4,5]. Dividing eq. (3) by eq. (4) yields

$$\frac{dr}{d\theta} = -r \frac{\left( L(r) \cos \theta + \frac{G(r)}{N_A} (f_A - N_R f_R) \right)}{M(r) \sin \theta}, \quad (13)$$

which was integrated backwards from a critical point at  $\theta = \pi$  and the separation for which the driving force pulling the two particles apart is balanced by the interparticle colloidal forces pulling them together [4].

Figures 5 and 6 show the collision efficiency as a function of the dimensionless driving force,  $N_A$ , at different double-layer thicknesses and strengths of the electrostatic repulsion. Apparently, electrophoretic aggregation is approximately an order of magnitude more efficient than buoyancy-driven aggregation. The stability region ( $E_{12} = 0$ ) for electrophoretic aggregation is shifted according to eq. (9), which is another manifestation that a given colloidal suspension is less stable to an applied electric field than to a gravitational driving force. As the driving force parameter increases, the collision efficiency at first increases as the electrophoretic or gravitational mechanisms pushing the particles together overcome the repulsive forces which hold them apart; a maximum is then reached, and further increases of the parameter  $N_A$  lead to a decrease in  $E_{12}$  because the attractive van der Waals forces required for aggregation become relatively weak. By comparing Figs. 5 and 6, it is seen that adding electrolyte to decrease the double-layer thickness (increase  $\kappa a$ ) enhances the collision rate. This is because attractive van der Waals forces become stronger relative to repulsive electrostatic forces as the double-layer becomes thinner and shields the repulsive surface charge.

## CONCLUSIONS

A suspension undergoing electrophoresis is less stable to aggregation than a comparable suspension undergoing gravity sedimentation. In addition, the collision efficiencies are much higher for electrophoretic aggregation than for gravity-induced aggregation. These results stem from the fact that electrophoretic motion is force-free and hydrodynamic interactions have a smaller effect on the relative motion than for gravity motion.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Smoluchowski, M. von. Versuch einer mathematischen theorie der koagulationskinetik kolloider Lösungen. *Z. Phys. Chem.* **92** (1903) 129.
2. Morrison, Jr., F.A. Electrophoresis of a particle of arbitrary shape. *J. Colloid Interface Sci.* **34** (1970) 210-214.
3. Todd, P & Hjertén, S. Free zone electrophoresis of animal cells I: Experiments on cell-cell interactions. in *Cell Electrophoresis*, ed. W. Schütt & H. Klinkman, Walter deGruyter & Co., Berlin (1985) pp. 23-31.
4. Davis, R.H. The rate of coagulation of a dilute polydisperse system of sedimenting spheres. *J. Fluid Mech.* **145** (1984) 179-199.

5. Melik, D.H. & Fogler, H.S. Gravity-induced flocculation. *J. Colloid Interface Sci.* 101 (1984) 72-83.
6. Batchelor, G.K. Sedimentation in a dilute polydisperse system of interacting spheres. Part 1. General theory. *J. Fluid Mech.* 119 (1982) 379-408.
7. Chen, S.B., & Keh, H.J. Electrophoresis in a dilute dispersion of colloidal spheres. *AIChE Journal* 34 (1988) 1075-1085.
8. Keh, H.J. & Yang, F.R. Particle Interactions in electrophoresis III: Axisymmetric motion of multiple spheres. *J. Colloid Interface Sci.* 139 (1990) 105-116.
9. Loewenberg, M. & Davis, R.H. Near-contact, electrophoretic particle motion. *J. Fluid Mech.* (1994, in press).
10. Jeffery, D.J., & Onishi, Y. Calculation of the resistance and mobility functions for two unequal rigid spheres in low-Reynolds-number flow. *J. Fluid Mech.* 139 (1984) 261-290.
11. Gregory, J. Approximate expressions for retarded van-der Waals interaction. *J. Colloid Interface Sci.* 83 (1981) 138-145.
12. Hogg, R., Healy, T.W., & Fuerstenau, D.W. Mutual coagulation interaction. *J. Colloid Interface Sci.* 83 (1981) 138-145.

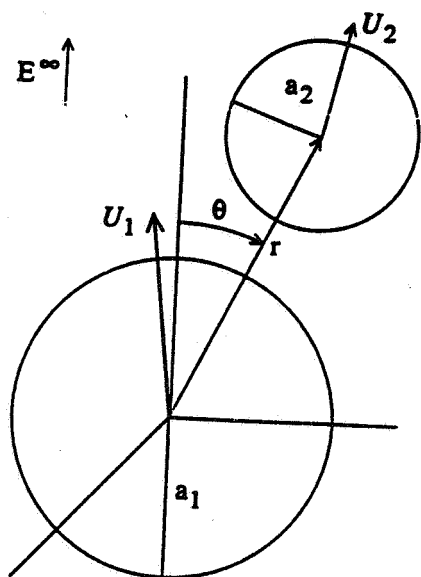


Fig. 1—Schematic of the relative motion of two charged spheres in an electric field.

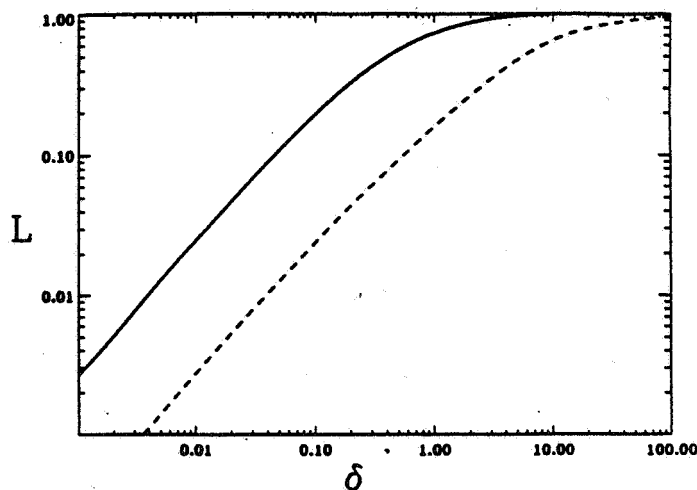


Fig. 2—Relative mobility function along the line of centers for gravity motion (dashed line) and electrophoretic motion (solid line) with  $\lambda = 2$ ,  $\gamma = 1$ , and  $\beta = 0.5$ .

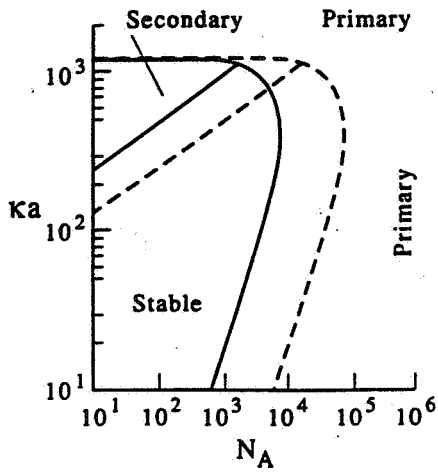


Fig. 3—Stability plot showing stable region, primary capture, and secondary capture for gravity-induced aggregation (dashed lines) and electrophoretic aggregation (solid lines) with  $\lambda = 0.5$ ,  $\gamma = 1$ ,  $\beta \rightarrow 1$ ,  $\nu = 0.1$ , and  $N_R = 400$ .

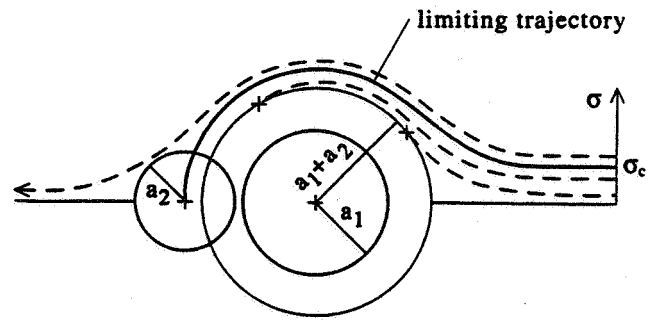


Fig. 4—Schematic of the relative trajectories of two spheres for different impact parameters;  $\sigma_c$  is the critical impact parameter defined such that aggregation occurs for  $\sigma < \sigma_c$  and not otherwise.

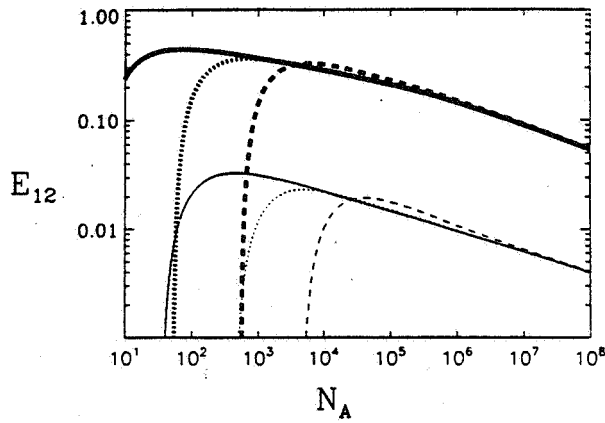


Fig. 5—The collision efficiency versus the ratio of driving force to attractive force for electrophoresis (thick lines) and gravity sedimentation (thin lines) for  $\lambda = 2$ ,  $\gamma = 1$ ,  $\beta = 0.5$ ,  $\nu = 0.044$ ,  $\kappa a = 10$ , and  $N_R = 10$  (solid lines), 100 (dotted lines), and 1000 (dashed lines).

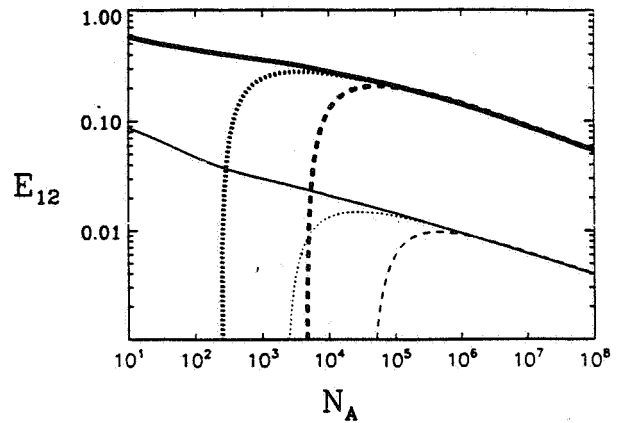


Fig. 6—The collision efficiency versus the ratio of driving force to attractive force for electrophoresis (thick lines) and gravity sedimentation (thin lines) for  $\lambda = 2$ ,  $\gamma = 1$ ,  $\beta = 0.5$ ,  $\nu = 0.044$ ,  $\kappa a = 100$ , and  $N_R = 10$  (solid lines), 100 (dotted lines), and 1000 (dashed lines).