The Pennsylvania State University

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Department of Chemical Engineering

CHARGE NONUNIFORMITY ON SUBMICRON PARTICLES

A Thesis in

Chemical Engineering

by

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ABSTRACT

Distribution of zeta potential on the surface of a colloidal particle has been previously modeled for spherical\textsuperscript{1}, ellipsoidal\textsuperscript{2} and spheroidal particles\textsuperscript{3}. The standard deviation in zeta potential distribution has been measured experimentally for micron sized spheroids using rotational electrophoresis. However, the zeta potential distribution has not been mapped on submicron sized and nano-sized particles experimentally since they are difficult to see using an optical microscope and since they are more susceptible to rotation due to Brownian motion and shear.

We have devised an experimental method to study the non-uniform distribution of zeta potential on sub-micron sized latex particles. We have observed that clusters of sub-micron particles which have a non-uniform distribution of surface charges rotate to orient themselves in an electric field. We have aggregated sub-micron sized particles using the stimulus-quenching technique\textsuperscript{4} and have used rotational electrophoresis to measure the angular velocity of the clusters in an electric field. A vertical rotational electrophoresis set up has been used to reduce shear due to settling of huge clusters. Angular velocity has been measured using video microscopy techniques.

Each cluster was then approximated to be a spheroid and fitted it to the existing spheroidal model proposed by Velegol and Feick\textsuperscript{3} to measure the non-uniformity on a patch of size L on the cluster. The non-uniformity was measured as the standard deviation from the mean zeta potential per patch. Charge non-uniformity was measured for both homo and hetero-clusters of particles.
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1. MOTIVATION AND RESEARCH GOALS

1.1 Motivation

Colloids find applications in the manufacture of a variety of commercial products like paints, cosmetics, paper and foodstuffs. At the core of every industrial process to manufacture colloids, there is a need to understand the stability behavior of the colloidal particles. A colloidal suspension that does not aggregate and settle down is considered to be stable over a period of time. For example, latex paints are complex formulations of a suspension of nanosized acrylic latex particles in an aqueous solution. They contain various components like micro-emulsions of polymeric binders, high concentration of inorganic pigment particles, small molecule surfactants, rheology modifying polymers, volatile organic cosolvents and plasticizers among other substances. Therefore, it becomes difficult to establish a common control handle over all the contradictory end use properties. One way to control the system at the molecular level is to control the stability by studying the surface charges on the particles.

Figure 1-1. Commercially used colloidal systems are complex with many interdependent variables.
Stability of the colloidal system is highly dependent on the individual contribution of the attractive and repulsive forces between the particles. The classical DLVO theory\(^1\) describes the electrostatic and van der Waals forces acting between two spherical particles in solution. One of the major assumptions is that the two particles carry a uniform charge over their surface. The attractive and the repulsive forces are then added to give the total potential which tells us about the energetics of the system.

Figure 1-2. Colloidal particles in solution repel each other due to electrostatic repulsion when their diffuse layers overlap.

The repulsive forces are taking to be of electrostatic origin and are dependent on the magnitude of the charge on the particle\(^1\). The charge on the surface of a particle corresponds to a potential called the zeta potential (\(\zeta\)) which drives the attraction or repulsion between particles. One of the most common ways to make a colloidal solution stable is by increasing the electrostatic repulsion between the particles so that they do not come near enough for the attractive forces to kick in. One of the ways electrostatic forces can be increased is by coating more polyelectrolyte on the particle surface\(^1\). The concentration of the polyelectrolyte on the surface directly corresponds to the charge carried by the particle.
The attractive forces are due to the ubiquitous van der Waals attraction between the two materials\(^8\). When the particles overcome electrostatic repulsion and come close enough, the induced dipole interactions attract the particles to each other. Though the electrostatic and van der Waals forces are the most studied in most colloidal systems, there are other forces like hydrophobic, solvation and steric forces etc that will be discussed later.

![Plot of total potential, electrostatic potential and van der Waals potential in terms of kT for two polystyrene spheres with ζ = -20 mV suspended in water at 293 K.](image)

As per the DLVO theory, particles that carry like charges should always repel each other. However, there have been instances where aggregates of particles of the same charge have been observed\(^{13,14}\). Thus, the assumption that the charges are uniformly distributed on the surface of the particle has been questioned. In 2000, Feick and Velegol\(^{15}\) experimentally observed and measured non-uniform distribution of charges using the technique of rotational electrophoresis\(^3\).
However, this technique could not measure the charge non-uniformity on particles that are difficult to see under the microscope.

Most industrially used colloids from paints to cosmetics are on the range of nano to sub-micron sizes. In order to study the effect of charge non-uniformity on stability for these systems, a method has to be devised to be able to experimentally measure the charge non-uniformity. Though non-uniformity has been studied for micron sized particles, a large part of the industrially used colloids are sub-micron to nano-sized particles. The stability behavior of the sub-micron sized particles is different from the micron sized ones. The primary reason is that they diffuse very rapidly, thus taking long periods of time to settle by gravity. It is more challenging to image them in a light microscope, and they become more responsive to shear than micron sized colloidal particles\textsuperscript{16}. Charge non-uniformity could lead to instability and the challenge here is to measure the charge non-uniformity on sub-micron sized particles that are hard to see. This thesis will also present techniques used to characterize stability and measure repulsive forces.

1.2 Research Goals

The thesis will attempt to answer the following questions which will pave way for understanding how charge non-uniformity can be controlled.

1) How can nonuniform charge distribution be measured experimentally on submicron sized latex particles? How to overcome difficulties in seeing the particles and due to shear rotation while studying submicron particles? How to modify the existing horizontal rotational electrophoresis set up to nullify shear effects?

2) How can we model nonuniform charge distribution on randomly shaped clusters of submicron particles?
Thus, this thesis aims to answer how charge non-uniformity on electrostatically stabilized sub-micron particles can be measured and modelled.

1.3 Overview of Thesis

Chapter 1 lays out the motivation and importance of answering how charge nonuniformity can be measured on submicron sized particles using rotational electrophoresis. The research questions are clearly laid out in this chapter.

Chapter 2 of the thesis gives an introduction to the field of colloidal physics and its fundamentals. It outlines the properties of colloidal systems, the primary forces acting among colloidal particles in solutions and techniques for measurement of these forces. This chapter briefly addresses stability of colloidal system and sets the basis for answering how charge nonuniformity affects the stability of colloidal particles.

Chapter 3 elaborates on the motivation and significance of measuring charge nonuniformity on submicron particles. It describes the difficulties in carrying out electrophoresis experiments using nanoparticles. It also paves way for describing how the difficulties in handling nanoparticles was tackled for carrying out rotational electrophoresis experiments.

Chapter 4 describes in detail the nature of particles used, their physical and chemical properties and the equipments used for carrying out the experiments. Control experiments and modification in experimental set up to nullify rotation due to shear have also been described in this chapter. Experimental results prior to analysis are shown and experimental terminologies are explained in this chapter.
Chapter 5 details how the Feick Velegol\textsuperscript{3} model for spheroidal electrophoresis was used for our system of randomly shaped aggregated. Analysis of raw data from rotational electrophoresis experiments have been explained and final results of charge nonuniformity on the cluster have been plotted.

Chapter 6 discusses the impact of measuring charge nonuniformity on submicron particles and emphasizes the contribution of this research to the field of colloidal science. The direction in which this research can be taken has been outlined and potential experiments are suggested.

**List of Symbols**

\begin{itemize}
  \item $\delta$ Distance between two particles in solution \ (nm)
  \item $\zeta$ Zeta potential \ (mV)
  \item $\Phi$ Potential energy of a system \ (J)
  \item $k$ Boltzmann constant \ $1.38 \times 10^{-23}$ J/K
\end{itemize}
2. INTRODUCTION

This chapter gives an introduction to the field of colloidal physics and its fundamental tenets. It outlines the properties of colloidal systems, the primary forces acting among colloidal particles in solutions and techniques for measurement of these forces. It briefly addresses stability of colloidal system and sets the base for answering how charge nonuniformity affects the stability of colloidal particles.

2.1 Properties of Colloidal Systems

A system is considered as colloidal if it contains particles in the range of less than one micron to several micrometers. Colloidal systems are also defined as substances that are uniformly dispersed through another phase while instability can be caused by changing the chemistry of the system. Every colloidal system consists of two phases, the dispersed phase and the dispersion medium and these can be solid, liquid or gas. As shown in Table 2-1, colloidal systems find applications extensively in day to day processes.

<table>
<thead>
<tr>
<th>Dispersion Medium</th>
<th>Gas</th>
<th>Liquid</th>
<th>Solid</th>
</tr>
</thead>
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<tr>
<td><strong>Dispersed Phase</strong></td>
<td>-</td>
<td>Foam</td>
<td>Solid foam</td>
</tr>
<tr>
<td>Gas</td>
<td>-</td>
<td>Vacuoles(^{17})</td>
<td>Pumice(^{19})</td>
</tr>
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<td></td>
<td>Fire extinguishers(^{18})</td>
<td>Styrofoam(^{20})</td>
</tr>
<tr>
<td>Liquid</td>
<td>Aerosol</td>
<td>Emulsion</td>
<td>Porous material</td>
</tr>
<tr>
<td></td>
<td>Clouds(^{21})</td>
<td>Fat digestion(^{22})</td>
<td>High impact plastics(^{24})</td>
</tr>
<tr>
<td></td>
<td>Hair spray</td>
<td>Drug delivery(^{23})</td>
<td>Opals(^{25})</td>
</tr>
<tr>
<td>Solid</td>
<td>Aerosol</td>
<td>Colloidal sol</td>
<td>Solid suspension</td>
</tr>
<tr>
<td></td>
<td>Volcanic smoke(^{26})</td>
<td>Bacteria(^{28})</td>
<td>Bone(^{30})</td>
</tr>
<tr>
<td></td>
<td>Solid pharmaceuticals(^{27})</td>
<td>Ink(^{29})</td>
<td>Composites(^{31})</td>
</tr>
</tbody>
</table>

Table 2-1. Types of colloidal systems and their applications
Colloidal assemblies can be found in all living systems in the form of cells, blood vessels, proteins, polyelectrolytes and amphiphilic molecules. Since many diseases occur in the colloidal scale, colloids play a huge role in targeted drug delivery and diagnosis. Thus, it becomes critical to understand the forces governing the properties of colloids. Brownian motion and light scattering are two important properties exhibited by colloids that have been exploited to explain many fundamental concepts behind colloidal particles. They are of utmost importance to our system as sub-micron sized colloidal particles are affected by Brownian motion to a greater extent than micron sized particles. Since they are difficult to see, light scattering is a viable technique to study rotation, stability and size distribution.

2.1.1 Brownian Motion

Brownian motion is the never ending random motion of colloidal particles suspending in a solution. Brownian motion results due to the continuous collision of the solvent molecules on the colloidal particle and this contributes to its random nature. There is no preferred direction in which the particle moves because there is no preferred way in which the solvent molecules can impinge on a particle. Therefore, there is equal probability for a particle to move in any of the three directions while undergoing Brownian motion.

However, the magnitude of the mean displacement depends upon the size and shape of the particle, the temperature of the environment and the viscosity of the solution. Smaller particles are easier to pushed around and affected by Brownian motion more than particles of larger radii. In our case of nanoparticle suspensions, Brownian motion is prevalent to a great extent and the effects of Brownian motion cannot be ignored. The thermal energy of the molecule at the given temperature is given by kT where k is the Boltzmann constant and T is the temperature in Kelvin. This thermal energy manifests as the kinetic energy that the particle possesses. Hence,
the mean displacement of a particle will be higher at higher temperatures. Similarly, if the particle is suspended in a highly viscous solution, the solvent molecules do not collide as much with the particle and hence the Brownian motion is not as appreciable. Brownian motion is also responsible for the diffusion of particles through a solution from a region of higher to lower concentration. This is the reason why smaller particles diffuse faster at higher temperatures in a gaseous suspension than bigger particles at the same conditions.

2.1.2 Light Scattering

In addition to Brownian motion, colloidal particles also scatter a photos when they are suspended in solution. This phenomenon is known as Tyndall scattering and it is dependent on the size of the particle and the wavelength of the incident light\textsuperscript{35}. There are five possible phenomena that might occur when a beam of light hits a colloidal particle – absorption, transmission, scattering, refraction and diffraction. For particles in the sub-micron and nano ranges light scattering is an the most important and used tool for measuring the particle size and sometimes even the particle orientation\textsuperscript{36,37}. Since nano particles are difficult to see under a microscope, detection and auto correlation of Brownian motion through light scattering is an accurate method to measure the diffusion coefficient\textsuperscript{38,39}. 


2.2 Colloidal Forces

Electrostatic Forces arise between two particles by virtue of their double layers. When a particle is suspended in solution, it develops a charge due to the dissociation of surface groups on it. This layer of fixed charges attracts a mobile layer of opposite charges from the solution. Though electroneutrality is maintained in the bulk, there arises a potential due to the containment of charges called the zeta potential within this electrical double layer (EDL) whose thickness is characterized by the Debye length ($\kappa^{-1}$)\(^{40}\). When two particles approach one another, their EDL overlap and this contributes to the interaction forces.

The electrostatic potential between two spherical particles can be derived by starting with the Poisson-Boltzmann distribution and then incorporating the Debye-Huckel equation and the
Derjaguin approximation as follows,

$$\Phi_{ES} = 2\pi \varepsilon a \psi_0^2 \exp(-\kappa x) \quad (2-1)$$

With the following boundary conditions:

The potential is constant at the surface of the particle,

$$x = 0, \psi = \psi_0$$

The zeta potential decays to zero in the bulk,

$$x \to \infty, \psi \to 0$$

More accurate results are available to describe electrostatic forces at low surface potentials, but for our system the surface potentials are high enough to use Equation 2-1.

### 2.2.2 van der Waals Forces

Van der Waals forces are always present between two particles suspended in solution because of the induced dipole between them when they come close enough. The van der Waals potential is given by,

$$\Phi_{VDW} = -\frac{A a}{12 x} \quad (2-2)$$

The Hamaker constant, A, depends on both the particles and the medium in which they are suspended. London calculated the potential of mean force per area $V^{VDW}$, between two infinite plates separated by a distance H as,

$$A_{eff}(x) = x \int_x^\infty \frac{A(h)}{h^2} \, dh \quad (2-3)$$

It should be noted here that the Hamaker constant is a function of the distance of separation between the particles. Since the van der Waals forces are caused due to a fluctuating dipole, retardation occurs as the frequency with which the electrons rearrange themselves is comparable to the speed of light. van der Waals attraction is of extreme importance to our system as the
instability caused due to charge non-uniformity is primarily because of low potential patches getting attracted to each other.

2.2.3 Hydrophobic Forces

Hydrophobic forces are developed when apolar molecules are introduced in an aqueous system. Since the apolar molecules do not favor bonding with the water molecules surrounding them, the water molecules structure themselves in layers of high energy compared to the apolar molecules. Therefore, the water molecules are effectively squeezed out of the distance between two apolar molecules, forcing them to escape into the bulk. This exclusion of the water molecules gives rise to the hydrophobic force\textsuperscript{46}. These forces are predominant in systems where bacterial adhesion is present\textsuperscript{47,48,49}.

2.2.4 Solvation Forces

Solvation forces are seen in systems where there is strong interaction between the solvent molecules with each other as well as with the solute molecules. This forces the solvent molecules, in our case the water molecules, to form a shell around the particle. The shell closest to the particle consists of water molecules that cannot interact with the bulk and this way, a gradient is set up from the surface of the particle to the bulk. The region where the effect due to shell formation on mobility and density is predominant is known as the solvation zone. The solvation force can be either attractive or repulsive depending on the shape and the interaction potentials. These forces are stronger than the van der Waals attraction at distances below 10 molecular diameters\textsuperscript{50}.

2.2.5 Depletion Forces

When most colloidal systems are synthesized by emulsion polymerization, there might be excess polymers in the form of polyelectrolytes and surfactants suspended in the solution. These non-

adsorbed polymers can cause a weak attraction among the particles when the interparticle distance becomes comparable to the length of the polymer. The polymers will then be squeezed out and a gradient of higher and lower polymer concentration will be set up. This gives rise to an osmotic pressure that causes the two particles to get attracted to each other. Though the depletion force is not as strong as van der Waals attraction or the solvation force, it becomes significant when there are no other attractive forces.

2.2.6 Steric Forces

When two particles come close enough for the polymer on their surface to overlap, steric repulsion may occur and push the particles away from each other. Essentially, the polymer hairs on the particles act as springs that bounce the particles off when they touch each other. This repulsive force is also driven by an osmotic pressure that arises due to the compression of the polymer chains. One of the major advantages of steric stabilization over electrostatic stabilization is that it not depends on the ionic strength of the solvent and works for both aqueous and non-aqueous media. However, in many cases the polymers on the surface that are used to electrostatically stabilize the system are also used for steric stabilization. Therefore, it is common for both these forces to be combined into a single entity and called the electrosteric forces.
Though there are various combinations of forces that exist between charged particles suspended in solution, we will concentrate on electrostatic and van der Waals forces in this thesis.

### 2.3 Colloidal Stability

As mentioned before, stability is the prime characteristic of a good commercial colloidal system. Stability depends on maintaining repulsion between the particles so that they do not aggregate and sediment when one particle comes into contact with another. Figure 2-3 illustrates the difference between a stable and unstable system.
The DLVO theory, independently developed by Derjaguin and Landau\textsuperscript{58} and Verwey and Overbeek\textsuperscript{59}, predicts the stability of a system by taking into account just the electrostatic and van der Waals forces. The DLVO potential is given by,

\[ \Phi_{DLVO} = \Phi_{ES} + \Phi_{VDW} \]  

Therefore, by definition, the mean force is,

\[ F = -\nabla \Phi_{DLVO} \]  

Derjaguin arrived at an approximate expression for calculating the electrostatic potential between two particles by treating each sphere as a series of concentric rings and then integrating the flat plate contribution for each ring\textsuperscript{60}

\[ \Phi_{ES} = \pi \varepsilon \left( \frac{a_1 a_2}{a_1 + a_2} \right) \left[ 2 \psi_1 \psi_2 \ln \left( \frac{1 + \exp(-k \xi)}{1 - \exp(-k \xi)} \right) + (\psi_1^2 + \psi_2^2) \ln \left( 1 - \exp(-2k \xi) \right) \right] \]  

Figure 2-3. Difference between a stable and unstable colloidal system
Similarly, the van der Waals potential for two spherical particles of different radii is given by the Hamaker expression,

$$\Phi_{VDW} = -\frac{A}{12} \left( \frac{y}{x^2 + xy + x} + \frac{y}{x^2 + xy + y} + 2 \ln \frac{x^2 + xy + x}{x^2 + xy + y} \right)$$  \hspace{1cm} (2-7)

Where,

$$x = \frac{\delta}{2a_1}$$  \hspace{1cm} (2-8)

$$y = \frac{a_2}{a_1}$$  \hspace{1cm} (2-9)

A common simulated result for calculating the stability ratio, W is given by,

$$W = 1 + 0.25 \left[ \exp \left( \frac{\Phi_{max}}{kT} \right) - 1 \right]$$  \hspace{1cm} (2-10)

However, the DLVO theory fails to predict the stability accurately when $|\zeta| < 50 \text{ mV}$ and this lays the basis of our research.

**2.4 Surfactants and Polyelectrolytes**

Surfactants are surface active reagents that consist of both a hydrocarbon and a polar region. Most surfactants have two components – a hydrophobic group and a hydrophilic group. The hydrocarbon group, which does not show affinity for water, is the hydrophilic group whereas the polar end is the hydrophilic group (Figure 2-4A). The hydrophilic group can be anionic, cationic, zwitterionic or non-ionic.

When surfactants are introduced into water, it would want to minimize its free energy. At low surfactant concentrations, they are soluble in water. When the concentration of surfactant increases above a certain level they start forming structures known as micelles (Figure 2-4B). Some of the surfactant molecules also partition at the interface (Figure 2-4C). The micelles form an exclusion region in the water phase at the critical micelle concentration (CMC). There is a
sharp change in conductivity and surface tension at the CMC. Although the shape of the micelle depends on the temperature, concentration and the nature of the hydrocarbon and the head group, they are usually spherical and distort with increase in concentration from the CMC.\(^6\)

![Figure 2-4. (A) Structure of a surfactant. (B) Structure of a micelle at CMC. (C) Surfactant monolayer at low concentrations.](image)

Surfactants are added to colloidal systems to modify the surface energetics of the particle.\(^6\) In consequence, the surfactant reduces the tendency of water to associate within itself and enables it to spread across the particle surface.

Another common component found in colloidal suspensions in addition to the surfactants is the polyelectrolyte. Polyelectrolytes have greater molecular weight than surfactants and they possess more charged groups. The number of charged groups on the polyelectrolyte is the same as the degree of polymerization. If the polyelectrolyte is highly branched, it can carry more charges as more end groups are exposed. The geometry of the polyelectrolyte is highly dependent on the ionic strength of the environment. At low ionic strengths, the end groups will repel each other they will have a rod like orientation. As the ionic strength increases, the effect of one end group on the other is screened and the polyelectrolyte assumes a coiled position (Figure 2-5A).
The most important function of the polyelectrolyte is to impart a charge on the colloidal particle. Polyelectrolytes are absorbed on the surface of the particle and obtain a charge when immersed in a solvent due to ionization of the end group (Figure 2-5B). Just like the surfactant, they can be positively, negatively or neutrally charged as well as exhibit a hydrophilic, hydrophobic or amphiphilic nature. Polyelectrolytes are so called because they are polymers which ionize like electrolytes but have one of their ions bound in the backbone and the other free in the solution.

![Figure 2-5. (A) Structure of a polyelectrolyte. (B) Polyelectrolytes impart charge to a colloidal particle.](image)

The stability of the colloidal system greatly depends upon the concentration of surfactant and polyelectrolyte present in it. Addition of surfactant increases the zeta potential thereby increasing the electrostatic repulsion. As advantageous as they may seem, surfactants are complex molecules that interfere with the downstream processing of the molecule. They are expensive and subsequently have to be removed from the final product. Polyelectrolytes are used industrially in stabilizing inks, paints, waste water treatment and paper manufacturing. However, the functionality of a polyelectrolyte is binary in that they are either stabilizing or
destabilizing depending on the concentration the particle is coated with. The destabilizing property of polyelectrolytes can be exploited in processes like waste water treatment where flocculation is induced to aid settling.

The absorption of surface additives directly affects the surface charge and surface charge controls stability to a great extent. The concentration of additives on the surface can be measured by various methods. Depletion methods like UV spectroscopy, high pressure chromatography and surface tension measurements quantify the depletion effect. Direct measurements like atomic force microscopy, NMR and x-ray diffraction measure the change in surface properties due to addition of additives. In this thesis, we use electrokinetic methods to quantify the electrophoretic mobility to measure the surface charge.

2.5 Significance of Zeta Potential

When a colloidal particle is suspended in solution, the surface groups on the particle dissociate and it acquires a charge on it. This layer of fixed charge attracts a mobile layer of opposite charges from the solution. Though electroneutrality is maintained in the bulk, a voltage is generated between these two layers of charges. This voltage is known as the zeta potential and the two layers of ions constitute the electrical double layer (EDL).

There are a variety of potentials used in the field of colloids like the surface potential ($\psi_0$), Stern potential ($\psi_S$) and the zeta potential as shown in Figure 2-6.
The surface potential is the potential at the surface of the particle ($\psi_0$). This cannot be measured by experimental methods. The Stern potential ($\psi_S$) is the potential at the Stern plane, which is the immobile layer of charges in the liquid that are immediate to the surface charges. This can also not be measured experimentally. The only quantity that is experimentally measurable is the zeta potential ($\zeta$) which is the potential of the double layer at the slipping plane. The zeta potential is the same as the Stern potential in most cases. The surface potential decays exponentially within the EDL and linearly within the slip plane.

The decay of the surface potential for a flat plate is given by the equation,

$$\psi = \psi_0 \exp(-\kappa x)$$  

(2-11)
An important conclusion we can make from Figure 2-6 is that, if we increase the thickness of the slipping plane by adsorbing a polymer onto the surface of the particle, the zeta potential will decrease.

The \( \zeta \) potential and the EDL are responsible of all of colloidal particles' properties. When we say two colloidal particles interact with each other, we actually mean the interaction between their EDLs. Thus, the \( \zeta \) potential and the EDL control assembly, aggregation and flow of colloidal particles\(^{69,70,71} \).

2.5.1 Measurement of Zeta Potential using Electrophoresis

Zeta potential of a suspension of particles can be measured using the technique of electrophoresis. Electrophoresis is the motion of a charged particle in an electric field (Figure 2-7).

![Figure 2-7. Electrophoresis of a negatively charged particle.](image)

The electrophoretic velocity \( (U) \) is given by the Smolchowski equation as,

\[
U = \frac{\varepsilon \zeta E_{\infty}}{\eta}
\]

(2-12)
Thus, if we know the applied electric field, the dielectric constant of the particle and the viscosity of the solution, we can measure the velocity the particle translates with to calculate its zeta potential. However, one of the major assumptions that the Smolchowski equation makes is that the particle is uniformly charged. Morrison showed that the Smolchowski equation is valid for a particle of any shape and the angular velocity of a uniformly charged particle is zero\(^7\). Therefore, the particle undergoes only translation and no rotation in an electric field.

\[ \Omega = 0 \] (2-13)

This assumption neglects the scenario where a particle may have patches of charges on it which gives rise to charge non-uniformity.

### 2.5.2 Correlation of Zeta Potential to Surface Charge

Though we keep talking about "charge" non-uniformity, the only quantity we can experimentally measure is the surface potential. Therefore, a particle that has a non-uniform surface charge distribution has a non-uniform surface potential distribution. The Poisson equation gives the relation between charge and potential as,

\[ \rho_S = \psi_0 \varepsilon \kappa \] (2-13)

We can integrate this result to obtain the following relationship for a spherical particle,

\[ \rho_S = \psi_0 \varepsilon \kappa \left( \frac{1 + \kappa a}{\kappa a} \right) \] (2-14)

In the above equation, \( \kappa \) is a very important parameter. \( \kappa^{-1} \) is known as the Debye length and it is effectively the thickness of the EDL. The surface potential decays exponentially and becomes negligible and close to zero after a few Debye lengths. In this thesis, the term "surface potential nonuniformity" is interchangeably used with "charge nonuniformity".
2.6 Origin of Zeta Potential Non-Uniformity

Traditionally, particles have been assumed to carry a uniform charge all over them. In other words, we can think of the particles of having the same charge at every point such that the same charge is smeared all over them as in Figure 2-8A. But it has been observed and measured experimentally that in reality colloidal particles have patches of charges on (Figure 2-8B) them which gives rise to non-uniformity\(^{73,74,75}\).

![Diagram](image)

Figure 2-8 (A) Uniformly charged particle. (B) Non-uniformly charged particle.

In the DLVO theory, the average zeta potential is a key factor in determining the stability. There are many cases in which the DLVO theory fails qualitatively\(^{76,77,78}\) and this might be due to the assumption that all particles are uniformly charged. Recently it has been postulated that non-uniform potential distribution can lead to attractive forces in systems that are electrosterically stabilized\(^{79,80}\). Ideally, we expect two particles that carry the same charge to repel each other and the system to be stable. This is only true when the charges are uniformly distributed. When charges are non-uniformly distributed, one of two things can happen. The first scenario (Figure 2-9A) is when two high potential patches face each other and the electrostatic repulsion is
dominant and the system stays stable. The second scenario is that two low potential patches face each other (Figure 2-9B) and this enables them to come close enough that van der Waals attraction kicks in. The particles aggregate due to the attraction and we see instability in systems we expect to be stable.

![Figure 2-9. (A) Electrostatic potential keeps the system stable when patches of high potential interact. (B) van der Waals attraction causes instability when patches of low potential interact.](image)

Charge non-uniformity has direct effect on predicting the stability of the system. For a system of colloidal-size particles, the average local zeta potential determines the stability of the particles and not the average zeta potential. Moreover, Brownian motion drives diffusion of the colloidal particles and an extreme zeta potential on a part of the particle's surface can affect its stability.
2.7 Previous Work in Modeling and Measuring Charge Non-uniformity

As mentioned earlier, the DLVO theory predicts stability of a suspension assuming that all particles are uniformly charged while the contrary has been observed. Experiments by Velegol et al.\(^1\) have shown that when mobility experiments indicate a high average zeta potential, the system shows instability. They have also showed that particles that are non-uniformly charged undergo rotation in addition to translation during electrophoresis. Grant and Saville found that heterogenously charged spheres spend more time in low energy orientations which means that attractive interaction potentials will dominate and the system will show instability\(^81\). Studies have been conducted on heterogenously charged spheres\(^82\) and plates and found that the attractive free energies are greater in magnitude than the attractive van der Waals forces. Koopal and van Riemsdijk\(^83\) found that a random distribution of charges results in an EDL different to that of homogenous surfaces. Gibb and Koopal confirmed this model with experiments on rutile-hematite systems\(^84\). Charge non-uniformity has been modeled for charged spheres with non-polarizable EDL by Anderson. Teubner\(^85\) and Yoon\(^86\) have extended the Anderson model for particles with large EDL. Solomentsev\(^87\) has extended his work to include polarizable EDL. Apart from spherical particles, Fair and Anderson have developed a model for the electrophoretic motion of non-uniformly charged ellipsoidal particles while Velegol et al. have developed a model to predict charge non-uniformity on spheroidal particles. Atomic force microscopy (AFM) is one of an oft used technique for measuring charge non-uniformity\(^88,89\). When the colloidal particle is attached to the cantilever and struck to a surface, the resulting force can be resolved into the electrostatic, van der Waals and the adhesion force between the particle and substrate. Though AFM seems like a suitable technique for measuring the surface forces, it has many shortcomings. The particle is fixed and does not undergo Brownian motion. And since the
particle is fixed to either a substrate or a centilever, only a portion of the particle is exposed for force measurement.

**List of Symbols**

- $k$: Boltzmann constant $\quad 1.38 \times 10^{-23} \text{ J/K}$
- $T$: Temperature $\quad (\text{K})$
- $kT$: Thermal energy
- $\kappa^{-1}$: Debye length $\quad (\text{nm})$
- $a$: Radius of the particle $\quad (\text{nm})$
- $\varepsilon$: Dielectric constant $\quad (\text{C}^2/\text{N-m}^2)$
- $A$: Hamaker constant $\quad (\text{J})$
- $W$: Stability ratio
- $\Psi_0$: Surface potential $\quad (\text{mV})$
- $E_\infty$: Applied electric field $\quad (\text{V/m})$
- $U$: Electrophoretic velocity $\quad (\mu\text{m/s})$
- $\eta$: Viscosity of the solution $\quad (\text{Pa.s})$
- $\Omega$: Electrophoretic angular velocity $\quad (\text{deg/s})$
- $\rho_s$: Surface charge density $\quad (\text{C/m}^2)$
3. CHALLENGES IN MEASURING CHARGE NONUNIFORMITY FOR NANOPARTICLES

Nanoparticles are also considered colloidal particles as they form a uniform suspension in solution and behave much like micron sized particles. In the past couple of decades, nanoparticles have gained prominence because of their widely recognized applications. They are prevalent in many commercial goods like surface coatings\textsuperscript{90}, adhesives\textsuperscript{91}, textiles\textsuperscript{92}, paints\textsuperscript{93} and cosmetics\textsuperscript{94}. One of the major difficulties in testing nanoparticles for charge non-uniformity is due to the difficulty in seeing them. There are other difficulties due to the virtue of their size like ease in undergoing rotation due to shear and changing planes due to Brownian motion.

This chapter elaborates on the motivation and significance of measuring charge nonuniformity on submicron particles. It describes the difficulties in carrying out electrophoresis experiments using nanoparticles. It also paves way for describing how the difficulties in handling nanoparticles were tackled for carrying out rotational electrophoresis experiments.

3.1 Resolution

The optical resolution of a microscope is defined as the minimum distance between two distinguishable radiating points. In other words, it is the minimum distance \( r \) that can be perceived between two points. The Rayleigh criterion\textsuperscript{95} gives a theoretical estimate of \( r \) as,

\[
r = \frac{\lambda}{2 \, NA}
\]

Where \( \lambda \) is the wavelength of the incident light and \( NA \) is the numerical aperture. The numerical aperture is a dimensionless number that characterizes the cone of light within which the lens can emit light. The numerical aperture depends on the refractive index of the medium the incident light traverses and the objective of the lens that is used. A lens with a large numerical aperture is
favorable since it will be able to capture the finer details by collecting more light.

\[ NA = n \sin \theta \]  

(3-2)

Another important criterion that affects the resolution is the depth of field (DOF). The DOF is the distance between the nearest and the farthest object that can be seen clearly in a microscope. If the depth of field is low and the size of the particle is small, the particle can move out of the plane and blur easily. Figure 3-1 illustrates the set up of an inverted optical microscope showing the numerical aperture and the depth of field.

![Numerical Aperture and Depth of Field](image)

Figure 3-1. Illustration of numerical aperture and depth of field of an inverted optical microscope.

For a 323 nm diameter nanoparticle, using a 100x oil objective, the resolution \( \delta \) is only around 315 nm (Figure 3-2B) as calculated in Appendix A.1. Moreover, the DOF is considerably low that only the particles that are at the lowest plane can be analyzed. This imposes a severe constraint on the z-direction and makes it unable to penetrate deeper to find particles closer to the middle plane.
Figure 3-2. Difficulty in seeing nanoparticles. (A) Micron-sized particles are easily discernible at low magnifications. (B) Sub-micron or nano-sized particles are barely discernible even at the highest magnification.

Though there are techniques like scanning electron microscopy (SEM), field emission SEM (FESEM) and atomic force microscopy (AFM) to image the particles, none of them capture the behavior of the particles in solution conditions, which is critical to us. Cryogenic SEM involves examining the sample by freezing it to temperatures as low as -100 °C to -175 °C against drying the sample on a silicon wafer. One of the major advantages of cryo-SEM is that the sample is maintained well below the glass transition temperature ($T_g$). When the particles are exposed to temperatures above the $T_g$, they start turning amorphous and fuse into a single film. However, we were not able to obtain high resolution images in which we could identify one particle from the other by this technique. Thus, the small size of the sub-micron particles imposes a hurdle in studying their non-uniformity.
3.2 Rotation Due to Shear

All particles undergo shear when they are enclosed in a channel. Shear stress arises due to the force vector component parallel to the cross section. For a Newtonian fluid flowing parallel to a plate, the shear stress is given by,

\[ \gamma(y) = \eta \frac{\partial u}{\partial y} \tag{3-3} \]

As seen from the above equation, the shear decreases with increase in distance from the wall. In the case of fluid between two semi-infinite walls, the shear is minimum at the geometric centre between the two walls as shown in Figure 3-3A.

![Figure 3-3](image)

Figure 3-3. (A) Shear and velocity profile of a fluid between two flat planes. (B) Rotation of a particle due to shear.

If a particle is suspended near the wall of the channel where the shear is considerably high, the top of the particle experiences a velocity slower than that at the bottom (Figure 3-3B). Due to this difference in velocities, the particle rotates to keep itself at equilibrium\(^9\). But at every point of its motion, the difference between these velocities is never zero and the particle continues to undergo rapid rotation. This unceasing rotation due to the presence of a shear field on an
elliptical particle is called Jeffery orbits.

The ellipsoids that rotate due to Jeffery orbits do so with a time period,

\[ T = \frac{2\pi}{\gamma} \left( \frac{r_e}{r_o} + \frac{1}{r_o} \right) \] \hspace{1cm} (3-4)

The time period of rotation is dependent on the size of the particle and nanoparticles rotate with a greater time period due to their smaller size.

### 3.3 Brownian Motion

As described in Chapter 2.1.1, Brownian motion is the continuous random motion of a colloidal particle as it is being pushed by solvent molecules on all sides. A particle has equal probability of moving in all three directions due to Brownian motion. The diffusion of a particle into a region of lower concentration is also due to Brownian motion. The Stokes-Einstein relation gives an expression to measure the diffusion coefficient (D) of a particle as,

\[ D = \frac{kT}{6\pi \eta a} \] \hspace{1cm} (3-5)

From Fick’s second law of diffusion, we know that,

\[ \frac{\partial n}{\partial t} = D \nabla^2 n \] \hspace{1cm} (3-6)

Solving this equation in the x-direction for the time interval \( \Delta t \),

\[ \langle \Delta x^2 \rangle = 2D\Delta t \] \hspace{1cm} (3-7)

Therefore, the total average displacement in all the three directions is,

\[ \langle \Delta x^2 + \Delta y^2 + \Delta z^2 \rangle = \langle \Delta \mathbf{x} . \Delta \mathbf{x} \rangle = 6D\Delta t \] \hspace{1cm} (3-8)

The average kinetic energy of a molecule over a long time is given by,

\[ \langle \frac{m(v_x^2 + v_y^2 + v_z^2)}{2} \rangle = \langle \frac{\mathbf{v} . \mathbf{v}}{2} \rangle = \frac{3kT}{2} \] \hspace{1cm} (3-9)
In addition to the translational Brownian motion, the particle also undergoes rotational Brownian motion when the solvent molecules shear the surface of the particle and impart a torque on it. The particle rotates due to an unbalanced torque as opposed to an unbalanced collision. The rotational diffusion coefficient is given by,

\[
D_R = \frac{kT}{8\pi\eta a^3}
\]  

(3-10)

It should be noted that the units on the rotational diffusion coefficient is \(s^{-1}\) which is different from that on translational diffusion coefficient which is \(m^2/s\).

Similar to translational Brownian motion, the average angle the particle rotates in a direction is given by,

\[
\langle \Delta \theta^2 \rangle = 2D_R \Delta t
\]

(3-11)

The rotational Peclet number is the ratio of the angular rotation to the rotational diffusion and it is given by,

\[
Pe_R = \frac{\Omega}{D_R}
\]

(3-12)

The angular velocity of a freely rotating doublet is given by,

\[
\Omega = \frac{\varepsilon(\zeta_2 - \zeta_1)E_0N_R}{2\eta a}
\]

(3-13)

The final form of the rotational Peclet number is,

\[
Pe_R = \frac{4\pi\varepsilon(\zeta_2 - \zeta_1)E_0N_R a^2}{kT}
\]

(3-14)
For our system of nanoparticles, the rotational Peclet is small enough (Appendix A.2) that there is a huge contribution due to the rotational diffusion coefficient. This, in combination with the large displacement due to Brownian motion and the difficulty to see the particles, are the major deterrents in measuring charge non-uniformity.

In this thesis, we propose an experimental technique to overcome these difficulties and enable us to measure the randomness in charge distribution. The next chapter outlines how we can make aggregates of nanoparticles that would increase the size of the particle undergoing electrophoresis. It also discusses how we can modify an existing model for spheroidal electrophoresis for micron sized particles and yet extract information about the charge nonuniformity on a submicron scale.

**List of Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$r$</td>
<td>Resolution</td>
<td>(nm)</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Wavelength of incident light</td>
<td>(nm)</td>
</tr>
<tr>
<td>$NA$</td>
<td>Numerical aperture</td>
<td></td>
</tr>
<tr>
<td>$n$</td>
<td>Refractive index</td>
<td></td>
</tr>
<tr>
<td>$\Theta$</td>
<td>Half angle</td>
<td>(deg)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Fluid shear</td>
<td>(N/m²)</td>
</tr>
<tr>
<td>$r_e$</td>
<td>Effective radius of the spheroid</td>
<td>(nm)</td>
</tr>
<tr>
<td>$D_R$</td>
<td>Rotational diffusion coefficient</td>
<td>(s⁻¹)</td>
</tr>
<tr>
<td>$N_R$</td>
<td>Coefficient of electrophoretic rotation</td>
<td></td>
</tr>
<tr>
<td>$E_0$</td>
<td>Applied electric field</td>
<td>(V/m)</td>
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</table>
4. EXPERIMENTAL METHODS AND RESULTS

This chapter describes in detail about the nature of particles used, their physical and chemical properties and the equipments used for carrying out the experiments. Control experiments and modification in experimental set up to nullify rotation due to shear have also been described in this chapter. Experimental results prior to analysis are shown and experimental terminologies are explained in this chapter.

4.1 Experimental Requirements

All experiments described in this thesis were performed to measure charge non-uniformity on submicron particles that are difficult to. The primary objectives of these experiments were to:

1. Develop a method for developing clusters of highly stabilized sub-micron particles of methyl methacrylate and butyl acrylate copolymer latex.

2. Set up a vertical rotational electrophoresis set up to measure angular velocity while clusters of amidine and sulfate polystyrene latex particles rotate in an electric field.

3. Use an approximation model to calculate the charge non-uniformity on sub-micron sized particles from the rotation data.

4.2 Colloidal Particles Used in Experiments

Three kinds of colloidal particles were used for all experiments. Polystyrene latex particles of 600 nm diameter and functionalized with sulfate groups (sPSL) and polystyrene latex particles of 400 nm diameter functionalized with amidine groups (aPSL) were purchased from Invitrogen, Inc (refer to Appendix B.4.1 for certificate of analysis). The latex spheres were found to be monodisperse when the size was measured through light scattering and were surfactant free. The sulfate and amidine groups are bound covalently to the latex surface. The zeta potentials were
measured using a Malvern Zetasizer equipment. The equipment measured the mobilities of $10^5$ particles and calculated the zeta potential and the standard deviation in zeta potential measurement using the Smolchowski equation. The physical properties of the amidine and sulfate polystyrene latex used in the experiments are listed in Table 4.1.

<table>
<thead>
<tr>
<th>Particle name</th>
<th>Particle Size (nm)</th>
<th>Zeta potential in DI (mV)</th>
<th>Density (kg/m$^3$)</th>
<th>Molecular weight (Da)</th>
<th>Batch Number</th>
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<tbody>
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<td>aPSL</td>
<td>400</td>
<td>39.6 ± 5.5</td>
<td>1055</td>
<td>~250000</td>
<td>#2672,1</td>
</tr>
<tr>
<td>sPSL</td>
<td>600</td>
<td>-42.7 ± 5.05</td>
<td>1055</td>
<td>~250000</td>
<td>#2199,1</td>
</tr>
</tbody>
</table>

The second kind of particle used was the acrylic paint latex particles provided by the Dow Chemical Company. All particles received consisted of a copolymer bead of methyl methacrylate (MMA) and butyl acrylate (BA) functionalized with methacrylic acid (MAA) which was covalently bound to the surface during the emulsion polymerization process (Figure 4-1).

Figure 4-1. Schematic representation of a MMA-BA latex particle in solution.
Five kinds of MMA-BA particles were used for experiments and their properties are summarized in Table 4-2. In addition to MMA, BA and MAA, the acrylic latex suspension also had surfactant SLS and rheology modifier HEUR. For all the experiments, the surfactant and HEUR in solution was removed by centrifuging the sample in a Sorvall Biofuge primo centrifuge at 4000 g for 20 minutes. The supernatent was drained and the particles were resuspended in DI water. This was repeated 6 times till the conductivity of the supernatent was comparable to that of DI water.

<table>
<thead>
<tr>
<th>% MAA (w/v)</th>
<th>Particle size (nm)</th>
<th>Zeta potential in DI (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>299.9</td>
<td>-67.5 ± 6.89</td>
</tr>
<tr>
<td>0.05</td>
<td>303.5</td>
<td>-62.5 ± 6.43</td>
</tr>
<tr>
<td>0.1</td>
<td>307.2</td>
<td>-67.2 ± 6.45</td>
</tr>
<tr>
<td>1</td>
<td>306.0</td>
<td>-66.9 ± 6.5</td>
</tr>
<tr>
<td>1</td>
<td>323.0</td>
<td>-71.1 ± 9.32</td>
</tr>
</tbody>
</table>

### 4.3 Aggregating Colloidal Particles using Stimulus-Quenching Technique

Since the nanoparticles are difficult to see, they were aggregated using the stimulus-quenching-fusing (SQF) technique (Figure 4-2). To summarize the technique, salt is added to shorten to Debye lengths and increase the van der Waals attraction. When sufficient aggregation is reached, the system is quenched by adding DI water to freeze aggregation. The system is quenched at the Smolchowski aggregation time given by,

$$\tau = \frac{\pi \eta a^3}{2 k T \varphi} W$$  \hspace{1cm} (4-1)
When the particles were highly stabilized, which was the case with the MMA-BA latex particles with 1% w/w of MAA on them, additional acid had to be added to increase the ionic strength of the bulk (Figure 4-3). Alternatively, a salt with a higher valence like calcium chloride or aluminum hydroxide could be added instead of a monovalent salt for the same effect.
The size of the aggregates can be controlled by controlling the rapid aggregation time. However, the resultant solution will have aggregates of all size ranges as aggregation is governed by the probability of one particle meeting the other, which is random (Figure 4-4).

![Figure 4-4: A normal distribution of the length of the aggregates at various quenching times. Aggregates were made of 400 nm aPSL and 600 nm sPSL particles at 1 mM KCl.](image)

4.4 Microscopy Techniques and Video Processing

Confocal microscopy was used to image various planes of the aggregate and build a z-stack of pictures into a video. A Leica TCS SP5 confocal laser scanning microscope (Figure 4-5) with 405 nm diode, 458/476/488/496/514 nm multiline Argon, 543 nm HeNe, 594 nm HeNe, and 633 nm HeNe was used. A resolution of 200 nm to 500 nm can be obtained using various objectives for this microscope.
In a confocal microscope, excitation light from a laser is absorbed by the fluorophores in the sample and it excites them to a higher vibrational energy state. Fluorescent light is given out once the fluorophores relax and reach the ground energy state again. This emitted light is collected by focused into a pinhole and amplified using photomultiplier tubes (PMT). At this stage, any out of focus light from the adjoining plane is cut off. The signals from the PMTs are constructed into an image and thus it becomes possible to image one plane at a time using confocal microscopy.

We imaged homo and hetero aggregates using confocal microscopy. For homo-aggregates of MMA-BA (Figure 4-6A) particles that did not have fluorophores embedded in them, we made the bulk solution fluorescent by adding Rhodamine-G red dye and adjusted the contrast to set the bulk to darkness.
Figure 4-6. (A) Homo-aggregate of MAA functionalized MMA-BA latex. (B) Hetero-aggregates of amine and sulfate functionalized PSL.

4.5 Measuring Angular Velocity through Rotational Electrophoresis

Electrophoresis is the motion of a charged particle in an electric field. When a particle has non-uniform patches of charges on it, in addition to undergoing translation, it undergoes rotation too. The electrophoresis cell (Figure 4-7B) consists of a 500 mm square glass capillary of inner side 0.9 mm which is fitted with two 0.5 mm gold wires that act as electrodes. The electrophoretic cell is mounted on a Nikon TE-3000 inverted optical microscope (Figure 4-7A) and an electric field is set up inside the cell using a voltage/current source from Keithley instruments (Figure 4-7C).
Figure 4-7. (A) Nikon TE 300 inverted optical microscope. (B) Electrophoretic set up of 0.9 x 0.18 x 1.2 mm capillary with gold electrodes. (C) Keithly source meter used for electrophoresis experiments

Experiments were carried at both constant current and constant voltage conditions. All videos were captured using a CCTV camera (QI click imaging camera from QImaging) and analyzed using Nikon NIS-Elements Advanced Research software package.

One of the problems faced while using a horizontal electrophoresis set up was finding an aggregate at the centre plane where shear is effectively non-existent. Heavier aggregates settled to the bottom of the capillary and since they had patches of positive charge on them, they adhered to the glass electrostatically (Figure 4-8A). Moreover, they acted as "icebergs" and induced hydrodynamic effects in planes we do not expect to see shear. Therefore, a vertical electrophoresis set up was adopted to increase the settling distance (Figure 4-8B).
Figure 4-8. (A) Horizontal electrophoresis set up where large cluster (circled) settles on the glass surface and acts as an iceberg. (B) In a vertical electrophoresis set up, the large cluster has more settling distance. The vertical electrophoresis set up can be achieved experimentally by tilting the microscope by $90^\circ$ on its back. Figure 4-9A shows the microscope tilted and Figure 4-9B shows the electrophoresis cell mounted vertically.

Figure 4-9. (A) The inverted optical microscope has been tilted to set up vertical rotational electrophoresis. (B) The electrophoresis cell has been mounted vertically on the microscope stage.
Prior to every experiment, the microscope was calibrated in the z-direction so that the particles that were analyzed did not rotate due to shear. Care was taken to keep the objective in focus within few micrometers of the centre plane or the plane of no shear. Similarly, the microscope was also calibrated in the x-plane so that there is no wall effect on the rotation of the particle. Figure 4-10 shows the planes in which particles were analyzed in.

Figure 4-10. The electrophoresis cell was calibrated in the x and z directions to avoid effects due to shear.

Once an electric field was set up within the electrophoresis cell, the particle rotated to orient itself by virtue of the non-uniformly distributed charges on its surface. The direction of the field was changed once the aggregate was seen to be aligned to the electric field. Figure 4-11 shows the rotation of the aggregate as it moves downwards during vertical rotational electrophoresis.

All the videos were analyzed using either NIS-Elements Advanced Research software from Nikon or Image J.

Figure 4-11. Panels showing rotation of aPSL-sPSL cluster under vertical rotational electrophoresis at different times.
4.6 Setting up control experiments

As shown in figure x, heteroaggregates exhibit nonuniform charge distribution whilst homoaggregates do not. Thus it follows that homoaggregates do not rotate in when the direction of the electric field is changed during electrophoresis though the translate in an electric field by virtue of their average zeta potential. For the purpose of a control, sPSL aggregates were formed using a similar stimulus-quenching technique. The aggregates were formed at 200 mM KCl and quenched to 10 mM KCl. The sample was then centrifuged to settle the aggregates down and the final salt concentration was maintained at 0.1 mM KCl. This was the same salt concentration at which aPSL-sPSL aggregates were observed in electrophoresis. Though the sPSL aggregates showed rotation during electrophoresis, none of the aggregates rotated exactly 180° when the field was reversed. Moreover, the rotation was random and preferred no particular axis. These rotations were attributed to shear flows and were recorded. The angular of rotation was measured using video analysis software on a scale of 0 to 180° and charge nonuniformity on the sPSL aggregates were calculated using the same model that was used for aPSL-sPSL aggregates.

List of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>η</td>
<td>Viscosity of the solution</td>
<td>(Pa.s)</td>
</tr>
<tr>
<td>a</td>
<td>Radius of the particle</td>
<td>(nm)</td>
</tr>
<tr>
<td>W</td>
<td>Stability ratio</td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>Boltzmann constant</td>
<td>$1.38 \times 10^{-23}$ J/K</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
<td>(K)</td>
</tr>
<tr>
<td>φ</td>
<td>Volume fraction</td>
<td></td>
</tr>
</tbody>
</table>
5. MODELLING ZETA POTENTIAL NON-UNIFORMITY

Chapter 5 details how the model for spheroidal electrophoresis was used for our system of randomly shaped aggregated. Analysis of raw data from rotational electrophoresis experiments has been explained and final results of charge nonuniformity on the cluster have been plotted.

5.1 Existing Models

The dependence of the shape of a particle on its zeta potential was first studied by Morrison in 1970. Morrison analytically derived that the shape of a particle does not have any effect on the average zeta potential. A key observation that he made in the paper was that the Smolchowski equation [eqn x] holds true for a particle with a uniform zeta potential.

\[ U = \frac{\varepsilon \zeta E_\infty}{\eta} \]  
\[ \Omega = 0 \]  

However, if the assumption that the particle has a uniform charge throughout its surface cannot be made, then the angular velocity during electrophoresis will not be zero.

One of the earliest analytical models for mapping the distribution of surface potential was proposed by Anderson for spherical particles while undergoing electrophoresis. The Smolchowski equation was modified to account for the nonuniform charge distribution by adding an angular velocity term. This angular velocity was by virtue of the particle acting as a dipole since it had a charge nonuniformity on its surface. Thus the fluid velocity at the particle surface was taken as the sum of the translational velocity due to the average surface potential and the angular velocity due to the standard deviation with which the average surface potential was distributed.

\[ \hat{v} = U + a\Omega \times n \]
Where $a$ is the radius of the particle and $n$ is the unit normal at the particle surface pointing into the fluid. Anderson's model related the angular velocity during electrophoretic rotation to the magnitude of electric field applied and the particle size to give the difference in potentials between different areas in the particle.

$$\Omega = \frac{9}{164\pi \eta a} (\zeta_1 - \zeta_2) \hat{e} \times \mathbf{E}_x$$  \hspace{1cm} (5-4)

Where $\hat{e}$ is the unit vector in the direction of the electric field. However, it is experimentally difficult to measure the angular velocity using the above model as a spherical particle is isotropic. An analytical model to measure the angular velocity during electrophoretic rotation for an ellipsoidal particle was proposed by Fair and Anderson. In this model, they defined two geometric tensors that accounted for the change in angular velocity with orientation of the ellipsoidal particles in an electric field.

Thwar and Velegol developed an analytical model for the effect of the standard deviation in zeta potential on colloidal interactions. In this paper, an expression for interaction of patches of charges between two flat plates was developed which was then extended to interaction of patches between two spheres. For two spheres of similar radii, the equation reduces to

$$\left( \frac{\sigma_A}{\langle \phi \rangle} \right)^2 = \frac{4\kappa R}{N} \left[ \left( \frac{\sigma_A}{\zeta_A} \right)^2 + \left( \frac{\sigma_B}{\zeta_B} \right)^2 + \left( \frac{\sigma_A}{\zeta_A} \frac{\sigma_B}{\zeta_B} \right)^2 \right]$$  \hspace{1cm} (5-5)

This expression gave a direct relation between the average zeta potential on each particle and the standard deviation with which it was distributed. Nevertheless, it was experimentally not feasible to measure the nonuniformity in zeta potential through electrophoresis using a model for spherical particles.
Feick and Velegol extended Anderson's model for charge distribution on an ellipsoid to a spheroidal particle. The key point in the model was that they approximated a doublet of two spheres to be a spheroid.

![Diagram showing approximation of doublet to spheroid](image)

Figure 5-1. Approximating two doublets with axes (a, a) doublets to spheroids with axes (2a, a, a).

The model further gives an expression for angular velocity in each direction due to the electric field in the corresponding direction by accounting for the geometric tensor $W$ that considers the particle orientation with respect to the electric field.

$$
\begin{bmatrix}
\langle \Omega_x^2 \rangle \\
\langle \Omega_y^2 \rangle \\
\langle \Omega_z^2 \rangle 
\end{bmatrix} = \left( \frac{\varepsilon}{\eta R} \right)^2 \frac{\sigma^2}{N \langle \Omega \rangle^2} \begin{bmatrix}
W_{1111} & W_{1212} & W_{1313} \\
W_{2121} & W_{2222} & W_{2323} \\
W_{3131} & W_{3232} & W_{3333}
\end{bmatrix} \begin{bmatrix}
E_x^2 \\
E_y^2 \\
E_z^2
\end{bmatrix}
$$

Feick and Velegol used this model to measure charge nonuniformity on micron sized doublets using rotational electrophoresis. They also measured the effect of additive adsorption on charge nonuniformity using this technique. In this thesis, aggregates of aPSL and sPSL particles formed using the stimulus quenching technique were approximated to be equivalent spheroids in similar experimental conditions. This chapter describes how the standard deviation in zeta potential distribution was calculated on individual submicron sized particles that went into the cluster by approximating the cluster as a spheroid.
5.2 Approximation of Aggregates as Spheroids

The aggregates of the aPSL and sPSL submicron particles formed by stimulus quenching technique were approximated to a spheroid by overlaying an equivalent ellipse with axes \((a, b)\) as shown in \textbf{Figure 5-2}. A two dimensional image of the aggregate was obtained from the inverted optical microscope. As it was not possible to determine the width of the aggregate, the third axes was approximated to be equal to the minor axes. Thus the aggregate was approximated to a spheroid with axes \((a, b, b)\).

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{spheroid}
\caption{(A) Schematic of a cluster of submicron particles bounded in a spheroid. (B) Schematic of a spheroid equivalent to the cluster.}
\end{figure}

\textbf{Figure 5-3B} shows a typical aggregate analyzed using image processing software. As illustrated in \textbf{Figure 5-3A}, the electric field is applied in the \(z\)-direction. The angle of initial orientation of the aggregate before the electric field is applied is denoted as \(\gamma\). The direction of dipole is denoted by the unit vector \(d\) while the normal through the focii is denoted by \(e\).
Figure 5-3. (A) Schematic of an aPSL sPSL aggregate approximated as a spheroid before an electric field has been applied. (B) Optical microscopy image of an aPSL sPSL aggregate at 20x that has been annotated for the major and minor axes and angle of orientation using image processing.

The angle of initial orientation of the aggregate is of importance as it governs the contribution of the net electric field on the aggregate. The electric field $E_0$ can be resolved into $E_z$ and $E_y$ components based on the angle of alignment ($\gamma$) of the aggregate to the direction of the dipole.

$$\overrightarrow{E_y} = \overrightarrow{E_0} \cos \gamma$$ \hspace{1cm} (5-7)

$$\overrightarrow{E_z} = \overrightarrow{E_0} \sin \gamma$$ \hspace{1cm} (5-8)

The Feick Velegol model takes into account the effect of aggregate rotation in all three axes due to applied electric field in all three axes. In our case, the aggregate rotates about the x-axis and therefore the angular velocity has magnitude only about the x-axis. Similarly, the electric field $E_0$ has components only in y and z direction.

$$\begin{bmatrix} \Omega_y^2 \\ \Omega_z^2 \\ \Omega_x^2 \end{bmatrix} = \left( \frac{\varepsilon}{\eta R} \right)^2 \begin{bmatrix} \sigma_z^2 \\ \sigma_z^2 \\ \sigma_x^2 \end{bmatrix} \begin{bmatrix} W_{1212} & W_{1313} \\ W_{3212} & W_{3313} \\ W_{2212} & W_{2313} \end{bmatrix} \begin{bmatrix} E_x^2 \\ E_y^2 \end{bmatrix}$$ \hspace{1cm} (5-9)
Therefore, the model reduces to the following equation,

$$\langle \Omega^2 \rangle = \left( \frac{\varepsilon}{\eta} R \right)^2 \left( \frac{\sigma^2}{N} \right) \left( W_{1212} E^2 + W_{1313} E^2 \right)$$

(5-10)

The values for the W tensor vary with the a/b ratio of the aggregate and are listed below in Table 5-1.

<table>
<thead>
<tr>
<th>a/b</th>
<th>W_{1212}</th>
<th>W_{1313}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.2424</td>
<td>7.9184</td>
</tr>
<tr>
<td>0.2</td>
<td>0.4972</td>
<td>7.0802</td>
</tr>
<tr>
<td>0.418</td>
<td>1.0326</td>
<td>5.0619</td>
</tr>
<tr>
<td>0.5</td>
<td>1.2013</td>
<td>4.3732</td>
</tr>
<tr>
<td>0.667</td>
<td>1.4624</td>
<td>3.1958</td>
</tr>
<tr>
<td>1.0</td>
<td>1.6875</td>
<td>1.6875</td>
</tr>
<tr>
<td>1.5</td>
<td>1.6380</td>
<td>0.7136</td>
</tr>
<tr>
<td>2.0</td>
<td>1.4819</td>
<td>0.3530</td>
</tr>
<tr>
<td>5.0</td>
<td>0.9745</td>
<td>0.0347</td>
</tr>
<tr>
<td>10.0</td>
<td>0.7817</td>
<td>0.0069</td>
</tr>
</tbody>
</table>

The W tensor was calculated numerically by discretizing the spheroid into a series of disks, placing point charges on them and accounting for pairwise interaction between point charges. In our case, all aggregates analyzed had a/b ratios between 1 and 3. When the exact value of a/b was not listed in Table 5-1, the required value was interpolated from the two nearest available values.
The final step in modifying the Thwar Velegol model was to define R as the effective length instead of 2b. In all the calculations, R was taken as follows,

\[ R = \sqrt{b^2 + a^2} \]  

The electric field \( E_0 \) was set up in the capillary using a constant current \( I_0 \). For the purpose of our calculations, \( E_0 \) was calculated from \( I_0 \) as follows,

\[ \frac{I_0}{\Lambda A} \]  

The unit of the electric field is V/m and this is equivalent to applying a constant voltage across the sample dividing it by the length of the capillary.

An important aspect of modeling charge nonuniformity on particles is fixing the patch size. The patch size is a length scale over which we expect to see nonuniform distribution of charges. The number of patches (N) depends on the patch size which in turn depends on the Debye length (\( \kappa^{-1} \)) of the particle. Contrary to what one might expect, a patch of charge cannot be seen under an optical microscope. It is a length scale we fix based on \( \kappa^{-1} \) to observe nonuniform charge distribution on the particle surface. According to Feick and Velegol, the number of patches varies inversely as \( \kappa^{-1} \),

\[ N = 8 \sqrt{\kappa R} \]  

If the salt concentration increases, the \( \kappa^{-1} \) decreases and the number of patches that can be observed increases.

It should be noted that the model can be used only over an ensemble of similar particles. Thwar and Velegol used similarly sized doublets of identical particles in the same electric field and experimental conditions. Each particle in the ensemble varied only in \( \gamma \) as the angle depends on rotational Brownian motion. In our case, all aggregates were similarly synthesized using the stimulus quenching technique at the same salt concentration of 0.1 mM KCl. The aggregates
varied only in size and $\gamma$. As shown previously in Figure 4-4, the size of the aggregate is randomly distributed with the quenching time. Hence, it was difficult to isolate clusters of identical or even approximately equal sizes.

5.3 Calculating the Angular Velocity of Rotating Aggregate

The video of the rotating aggregate was analyzed using Nikon image processing software. The aggregate was tracked manually from when the electric field was applied till it aligned to the electric field. Once the aggregate aligned to the electric field, the direction of the field was reversed. The reversed electric field was held till the aggregate aligned to it and the process was repeated one more time. The angular velocity was measured by first fixing two points marking the major axis of the particle (as shown in Figure 5-4). The coordinates of the points were obtained as $(x_1, y_1)$ and $(x_2, y_2)$ for every 100 frames. From the two points, the angle between them was calculated using the two-point formula,

$$\theta = \tan^{-1} \left( \frac{y_2 - y_1}{x_2 - x_1} \right)$$

Figure 5-4. Panels showing the method of calculating angle rotated between times $t_1$ and $t_2$. Two points were marked across the major axis of the spheroid and $\tan \Theta$ was calculated.

It should be noted that the angle $\Theta$ obtained varies between -90° and +90° as it is calculated as the arc of a tangent. These angles were translated to a 0 to 180° scale by adding 180° to angles
Figure 5-5. Experimental data obtained by recording the rotation of a cluster with axes (23.97 μm, 8.8 μm) in an electric field of 1260 V/m at 0.1 mM KCl.

The angular velocity was then calculated between two successive rotations as,

\[ |\Omega| = \frac{|\theta_{i+1} - \theta_i|}{t_{i+1} - t_i} \]  \hspace{1cm} (5-15)

The angular velocity keeps changing with the orientation of the aggregate. There are two important effects that peak the angular velocity of the aggregate. When one part of the aggregate is heavier than the other, the aggregate rotates rapidly due to gravity and this peaks the angular velocity due to gravitational rotation. Similarly, there could also be an electrophoretic rotation component that increases the angular velocity due to charge segregation. The following two phenomena are discussed below in relation to our system.
5.3.1 Gravitational Rotation

Gravitational rotation can be estimated by approximating two sections of the aggregate as two spheres with one sphere heavier than the other as shown in Figure 5-6.

![Figure 5-6](image)

Figure 5-6. (A) Schematic of an aggregate having one half heavier than the other. Each half has been represented by circles of differing radii. (B). Optical microscope image of an aggregate that has been approximated to a doublet made of singlets varying in size.

Since sphere 1 is heavier than sphere 2, sphere 1 will be pulled down more than sphere 2 is thereby causing the cluster to rotate at a high angular velocity till it orients itself. This angular velocity scales as the difference in the translational velocities of these two spheres and the length of the aggregate.

\[ |\Omega_g| \sim \frac{|u_2 - u_1|}{L} \]  \hspace{1cm} (5-16)

Using Stokes law of settling under gravity,

\[ u = \frac{2 a^2 (\rho_p - \rho_f)}{9 \eta} g \]  \hspace{1cm} (5-17)

Since L is the centre to centre distance,

\[ L = a_1 + a_2 \]  \hspace{1cm} (5-18)
We arrive at an expression for gravitational angular velocity as,

\[ |\Omega_G| = \frac{2 (\rho_p - \rho_f) |a_2 - a_1|}{\eta} g \]  

(5-19)

5.3.2 Electrophoretic Rotation

The aPSL-sPSL clusters were formed by the stimulus quenching technique and were aggregated at the secondary minima in the DLVO plot (Figure 1-3). This means that each submicron size particle is not irreversibly adhered to the other. For doublets undergoing electrophoresis, each of the spheres rotate in addition to the whole doublet rotating since they are still separated by a small distance. The rotation coefficient \( N_R \) is dependent on the ratio between the particle size and distance of separation. For \( \delta/2a \geq 1 \), \( N_R = 0.64^{100} \). When there is no electric field applied the particle is randomly oriented and the charges on the particles are randomly oriented too (Figure 5-7A). Once the electric field is applied, the individual submicron particles start segregating due to electrophoretic rotation (Figure 5-7B) and by the time equilibrium is reached positively charged particles are segregated from the negative (Figure 5-7C). The electrophoretic angular velocity is given by,

\[ |\Omega_E| = \frac{\varepsilon (\rho_p - \rho_f) E_0 N}{2\eta a} \]  

(5-20)
Figure 5-7. (A) Schematic of an approximated spheroid that is randomly oriented at t=0 when no electric field is applied. (B) The charges in the spheroid partition to form a dipole once an electric field has been applied since the cluster was formed in a secondary minima. (C). The dipole orients itself in the electric field at a time \( t_e \).

Though the angular velocity is affected simultaneously by gravitational and electrophoretic contributions, the cluster will rotate at the maximum angular velocity when its dipole is perpendicular to the electric field. Thus the absolute maximum angular velocity which contains the effect of rotation due to gravity and electric field is taken to calculate the charge nonuniformity.

### 5.4 Calculating Standard Deviation of ζ Distribution

The absolute maximum angular velocity that was obtained from analysis of the video was used in equation-x to arrive at a value for standard deviation in zeta potential per patch, 

\[
\frac{\sigma_\xi^2}{N} = \frac{|\Omega_\xi|^2}{\left(\frac{\xi}{\eta R}\right)^2 \left(W_{1212}E_2^2 + W_{1313}E_2^2\right)}
\]  

(5-21)

The same equation was used to calculate the standard deviation in zeta potential distribution per patch on sPSL aggregates. Figure x shows the variation in the distribution of potential per patch.
between aPSL-sPSL system and the control sPSL system.

Figure 5-8. Deviation of zeta potential per patch in the aggregate against the aggregate number. The ● markers denote aPSL sPSL aggregates and the ○ represents the results from the sPSL clusters.

Multiplying by the number of patches N, we can get a value for $\sigma_\zeta$ between one patch and the other. As can be seen in Figure 5-9, the standard deviation in zeta potential is only in the order of a few mV for sPSL aggregates.
Figure 5-9. Standard deviation in zeta potential distribution of each aggregate. The ● markers denote aPSL sPSL aggregates and the ○ represents the results from the sPSL clusters.

**List of Symbols**

- \( \zeta \)  Zeta potential (mV)
- \( k \)  Boltzmann constant \( 1.38 \times 10^{-23} \) J/K
- \( \varepsilon \)  Dielectric constant \( (\text{C}^2/\text{N-m}^2) \)
- \( U \)  Electrophoretic velocity (μm/s)
- \( E_\infty \)  Applied electric field (V/m)
- \( \eta \)  Viscosity of the solution (Pa.s)
- \( \Omega \)  Electrophoretic angular velocity (deg/s)
- \( \Phi \)  Potential energy of a system (J)
\( \sigma \)  Standard deviation in \( \zeta \) distribution  (mV)

\( a \)  Major axis of spheroid  (nm)

\( b \)  Minor axis of spheroid  (nm)

\( R \)  Equivalent radius of spheroid  (nm)

\( N \)  Number of patches of nonuniformity

\( L \)  Length scale of observation  (nm)

\( \gamma \)  Initial orientation angle  (deg)

\( I_0 \)  Current across the electrophoretic cell  (\( \mu \)m)

\( \Lambda \)  Conductivity of the solution  (\( \mu \)S)

\( A \)  Area of cross section of capillary  (mm\(^2\))

\( \Theta \)  Instantaneous angle in applied field  (deg)

\( \Omega \)  Angular velocity during electrophoresis  (deg/s)

\( \rho_p \)  Density of the particle  (kg/m\(^3\))

\( \rho_f \)  Density of the fluid  (kg/m\(^3\))

\( \delta \)  Distance of separation between particles  (nm)

\( N_R \)  Coefficient of electrophoretic rotation
6. CONCLUSIONS AND FUTURE WORK

This chapter discusses the impact of measuring charge nonuniformity on submicron particles and emphasizes on the contribution of this research to the field of colloidal science. The direction in which this research can be taken has been outlined and potential experiments have been suggested.

6.1 Impact of Work

The work presented in this thesis contributes significantly to the understanding of charge distribution on the surface of colloidal particles. The primary contributions are as follows:

1. Developed an experimental technique for measuring standard deviation in surface potential distribution on submicron sized particles. This technique further reinforced the observation that a uniformly charged particle will not rotate during electrophoresis to align itself when the direction of the electric field is changed. Using this as the control experiment, the angular velocity of rotation of nonuniformly charged clusters were measured as an indicator of charge nonuniformity. The advantages of this technique include simplicity in experimental set up, ease of carrying out the experiment and ease of recording and analyzing data (Chapter 4).

2. Approximated existing spheroidal model to calculate charge nonuniformity for randomly shaped clusters of submicron particles. The approximation model uses the angular velocity of the rotating clusters to indicate the charge nonuniformity per patch of each cluster. Each cluster was imaged in 2 dimensions as (a, b) and approximated to a spheroid of 3 dimensions (a, b, b) and the standard deviation in zeta potential was calculated. (Chapter 5)
6.2 Future Work

6.2.1. Coating submicron particles on to micron sized doublets to calculate charge nonuniformity

A better fit to the spheroidal model can be obtained by coating submicron sized particles on to a doublet formed by the stimulus quenching technique. A two step coating process can be carried out where the formation of a homodoublet takes place in a high salt concentration (around 200 mM KCl) at the first step. The second step consists of submicron particles of charge opposite to the homodoublet adhering to doublet at a lower salt concentration (around 1 mM KCl) as shown in Figure 6-1.

Figure 6-1. (A) Stage (1) of the process consists of forming a homodoublet at a high salt concentration. (B) Stage (2) of the process consists of partially coating the micron sized doublet with submicron sized particles at a low salt concentration to emulate a nonuniformly charged particle. (C) FESEM image of a doublet of 3.3 μm aPSL particles coated with 200 nm sPSL particles.

For submicron particles that have a charge nonuniformity in the nanometer scale, the submicron particles can be completely coated on the micron sized doublet. The effect of the surface potential of the doublet will be nullified as the surface potential decays exponentially,

$$\psi = \psi_0 \exp(-\kappa x)$$  \hspace{1cm} (6-1)

Thus within a few Debye lengths, the surface potential of the micron size doublets would have
reduced to zero. This technique holds true when the radius of the submicron particle is much greater than the Debye length. Positive and negative control experiments would be to record angular velocity of uncoated doublet and a doublet completely coated with submicron particles of uniform charge.

6.2.2. Studying the effect of charge nonuniformity in the presence of organic additives

Though our model system is surfactant free and consists of latex particles suspended in DI water, industrial systems have many organic additives that can greatly change the charge nonuniformity on the surface of the particles. As shown in Figure 1-1, industrial colloidal systems typically have surfactants and rheology modifiers besides other additives. The commonly used surfactants are sodium laureth sulfate (SLS) or sodium dodecyl sulfate (SDS). Since both SDS and SLS are anionic surfactants, the hydrophobic end of the molecule adheres to the latex particle free of functionalization and the hydrophilic end remains negatively charged (Figure 6-2). Thus this levels out the charges on the particle surface and kills charge nonuniformity. Moreover the additional Na\(^+\) ions released into the bulk due to the ionization of the surfactant contributes to the screening of ions and affects the mean zeta potential of the particle.
Figure 6-2. (A) A latex surface that is coated nonuniformly with polyelectrolytes that will exhibit a nonuniform zeta potential distribution. (B) When a surfactant is added to the nonuniformly charged latex surface, it adheres to the surface and kills charge nonuniformity.

Hydrophobically modified ethoxylate urethanes (HEUR) are commonly used rheology modifiers. HEURs consist of a polyethylene chain backbone with hydrophobic end groups. HEURs change the hydrophobicity of the particle surface and hence affect the charge nonuniformity. One way to study the effect of HEURs and surfactants on charge nonuniformity is to add varying concentrations of a nonionic surfactant like Triton-X 100 to the system that will displace HEURs and SDS from the surface of the particle. Since Triton-X 100 adhered to the particle surface by virtue of its hydrophobicity and does not ionize in solution, it does not affect the charge nonuniformity.
6.2.3. Measuring repulsive forces between submicron sized particles during settling at high gravities

Electrosterically stabilized particles as shown in Figure 2-2 are kept stable by electrostatic as well as steric stabilization. The repulsive force one particle exerts on the other can be measured as a function of the acceleration due to gravity it is being spun at as well as the concentration of the system. Measuring the repulsive forces between submicron particles find importance in calculating the stability of the colloidal system. The system becomes unstable when the repulsive forces are overcome.

![Diagram](image)

**Figure 6-3.** 1-dimensional schematic of a system of submicron sized latex particles settling under gravity on a negatively charged glass plate.

A freely settling particle in solution (Figure 6-3) experiences electrostatic repulsion, gravitational force and van der Waals attraction. The total potential of the particle as a function of distance from the glass surface can be calculated by the equation,

\[
\phi(h, n) = 2 \pi \varepsilon a \psi^2 \exp(-kh) + n \frac{4}{3} \pi a^3 (\rho_p - \rho_f) gh - \frac{A a}{12 h} \tag{6-2}
\]

The minimum of the potential or the point at which the force is zero gives us the equilibrium
height at which the particle will be found above the glass surface. The Boltzmann distribution gives the probability of finding a particle of a giving energy at a given height,

\[ P(h) = \frac{\int_{h_1}^{h_2} \exp\left(-\frac{\phi(h,n)}{kT}\right) dh}{\int_{0}^{L} \exp\left(-\frac{\phi(h,n)}{kT}\right) dh} \quad (6-3) \]

Ensemble averaging over the length of settling L gives us the mean height of maximum approach and the deviation from the mean height.

\[ \langle h \rangle = \frac{\int_{hm_{\text{max}}}^{h_0} h_0(n)P(h,n)dh}{L} \quad (6-4) \]
\[ \langle h^2 \rangle = \frac{\int_{hm_{\text{max}}}^{h_0} h_0(n)^2 P(h,n)dh}{L^2} \quad (6-5) \]
\[ \sigma_h^2 = \langle h^2 \rangle - \langle h \rangle^2 \quad (6-6) \]

By extending the model, electrolyte concentration, stabilizer concentration and the effect of the neighboring particles in the x and z directions as a function of time can be accounted for. The model can be validated by experiments on sulfate polystyrene sub-micron sized latex particles which will carry no charge nonuniformity. These experiments can be compared against an acrylic paint latex system with different concentrations of stabilizer on them.
## List of Symbols

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<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
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<tr>
<td>a</td>
<td>Major axis of spheroid</td>
<td>(nm)</td>
</tr>
<tr>
<td>b</td>
<td>Minor axis of spheroid</td>
<td>(nm)</td>
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<tr>
<td>$\Psi_0$</td>
<td>Surface potential</td>
<td>(mV)</td>
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<td>$\kappa^{-1}$</td>
<td>Debye length</td>
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<td>n</td>
<td>Number of particles in the system</td>
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<td>$h_0$</td>
<td>Equilibrium height of approach</td>
<td>(nm)</td>
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<td>g</td>
<td>Acceleration due to gravity</td>
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<td>$\rho_P$</td>
<td>Density of the particle</td>
<td>(kg/m³)</td>
</tr>
<tr>
<td>$\rho_F$</td>
<td>Density of the fluid</td>
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<td>$\varepsilon$</td>
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<td>$\Lambda$</td>
<td>Hamaker constant</td>
<td>(J)</td>
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<td>$\Phi$</td>
<td>Potential energy of the system</td>
<td>(J)</td>
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</table>
APPENDIX A: SAMPLE CALCULATIONS

A.1 Calculation of Resolution Delta

Resolution delta,

\[ \delta = \frac{\lambda}{2 \cdot NA} \]

Where \( \lambda \) is the wavelength of incident light and \( NA \) is the numerical aperture.

\[ NA = n \sin \theta \]

Where \( n \) is the refractive index of the medium and \( \theta \) is the half angle.

For a plan fluorite 100x oil objective,

\( n = 1.5 \)

\( \theta = 72.1^\circ \)

\[ NA = 1.5 \sin (72.1) \]

\[ NA = 1.427 \]

\( \lambda = 900 \text{ nm} \)

Therefore,

\[ \delta = \frac{900 \times 10^{-9}}{2 \times 1.427} = 315 \text{ nm} \]

The smallest discernible distance between two points in a plan fluorite 100x oil objective is 315 nm.

A.2 Calculation of Rotational Peclet Number

\[ Pe_R = \frac{4 \pi \varepsilon (\zeta_2 - \zeta_1) E_0 N a^2}{kT} \]

\( \zeta_2 \) – zeta potential of sPSL (mV)

\( \zeta_1 \) – zeta potential of aPSL (mV)
E₀ – applied electric field (V/m)

Nᵣ – electrophoretic rotation coefficient

E – dielectric constant (C²/N-m²)

a – radius of the particles (nm)

k – Boltzmann constant (J/K)

T – temperature (K)

\[ Pe_R = \frac{4 \pi \times 7.1 \times 10^{-10} \times (70 - (-40)) \times 10^{-3} \times 0.4 \times (150 \times 10^{-9})^2}{1.38 \times 10^{-23} \times 293} = 0.0218 \]
A.3 Calculation of Debye Length

$$\kappa^2 = \frac{2 Z^2 e^2 c_\infty}{\varepsilon k T}$$

$Z$ - Valence of the salt

$e$ - Charge of electron (C)

$c_\infty$ - Concentration of salt solution (#/m$^3$)

$\varepsilon$ – Dielectric constant of the solution (C$^2$/N-m$^2$)

$k$ – Boltzmann constant (J/K)

$T$ – Temperature (K)

$$\kappa^2 = \frac{2 (1)^2 (1.6 \times 10^{-19})^2 (0.1 \times 6.023 \times 10^{23})}{(7.1 \times 10^{-10})(1.38 \times 10^{-23})(293)} = 1.07418 \times 10^{15}$$

$$\kappa^{-1} = 30.5 \text{ nm}$$
A.4. Calculation of Settling Time

According to Stokes law,

\[ U = \frac{2 \alpha^2 (\rho_p - \rho_f)}{9 \eta} g \]

Where,

- \( U \) – settling velocity (m/s)
- \( a \) – radius of the particle (m)
- \( \rho_p \) – density of the particle (kg/m\(^3\))
- \( \rho_f \) – density of the fluid (kg/m\(^3\))
- \( \eta \) – viscosity of the fluid (kg/m-s)
- \( g \) – acceleration due to gravity (m/s\(^2\))

Thus it takes around 55 minutes to settle 300 nm sPSL particles by centrifuging them at 1000 g.
A.5 Calculation of Number of Patches of Non-uniformity

Figure A-1. Optical microscope image of a randomly oriented aggregated in which initial angle and the semi axes have been marked.

\[ \kappa^{-1} = 30.5 \text{ nm} \]

\[ a = 24.97 \mu m \]

\[ b = 9.93 \mu m \]

The equivalent radius

\[ R = \sqrt{a^2 + b^2} = 26.872 \mu m \]

The length scale of observation

\[ L = \sqrt{R \kappa^{-1}} = 905.31 \text{ nm} \]

The number of patches being observed

\[ N \sim 8 \kappa R = 7048.39 \]
APPENDIX B: VENDORS

B.1 Particle Vendors

B.1.1 Life Technologies™

Surfactant free sulfate polystyrene latex particles, 600 nm, 8% w/w

Surfactant free amidine polystyrene latex particles, 400 nm, 4% w/w

Surfactant free amidine polystyrene latex particles, 3300 nm, 4% w/w

Invitrogen Technologies

3175 Staley Road
Grand Island, NY 14072 USA

B.1.2 Dow Chemicals

0.01%, 0.05%, 0.1%, 1% w/v MAA coated 300 nm MMA-BA latex particles

Dow Spring House Technical Centre

727 Norristown Road

Spring House, PA 19477

B.2 Equipment Vendors

Nikon TE 300 Eclipse Inverted Microscope

Optical Apparatus Company

136 Coulter Avenue

Ardmore, PA 19003
Model 2410 Source meter
Keithley Instruments, Inc.
28775 Aurora Road
Cleveland, OH 44139

Malvern Zeta Sizer
Malvern Instruments Inc
117 Flanders Road
Westborough
MA 01581-1042

0.9 x 0.18 x 1.26 mm borosilicate glass capillary tubes
Vitro Com Inc.
P.O. Box. 125
Mountain Lakes, NJ 07046

B.3 Chemical Vendors

Sulfuric acid, 95-98%, reagent grade

Potassium chloride, BioXtra, > 99.0%
Sigma Aldrich (R)
PO Box 14508
St. Louis, MO 63178
B.4.1 Certificate of Analysis for aPSL particles

Molecular Probes®

Certificate of Analysis

Catalog Number: A37474
Product Name: Amidine latex, 4% w/v 0.4 μm
Appearance: white suspension
Medium: distilled, de-ionized water
Lot Number: 54235A

Positively charged polystyrene microspheres with amidine functional groups on the surface. Surface charge is pH dependent. Stable at low pH. Avoid using multivalent anions. Avoid using glass vessels. Surface is hydrophobic in nature. SONICATE WELL BEFORE USE. STORE AT 4°C, DO NOT FREEZE

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<th>INTERFACIAL DYNAMICS</th>
<th>LOT DATA</th>
<th>SPECIFICATION</th>
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<td>Batch/Material Number</td>
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<td>Mean Diameter¹</td>
<td>0.40 μm</td>
<td>0.4 ± 0.05 μm</td>
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<td>Standard Deviation of Diameter</td>
<td>0.013 μm</td>
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<td>Coefficient of Variation of Diameter</td>
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<td>≤7.0 %</td>
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<td>Percent Solids w/v</td>
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<td>3.5 - 4.5 %</td>
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<td>Particle Number Per Milliliter of Latex</td>
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<td>n.a.</td>
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<td>Specific Surface Area</td>
<td>1.4 x 10^5 cm²/g</td>
<td>n.a. cm²/g</td>
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<tr>
<td>Density of Polystyrene at 20°C</td>
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<td>1.056 g/cm³</td>
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<td>Refractive Index of Polystyrene at 589 nm, 20°C</td>
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<td>1.591</td>
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<td>Charge Content²</td>
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<td>Charge Groups per Particle</td>
<td>6.0 x 10⁵</td>
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1. Determined from transmission electron microscopy.
2. Measured from conductometric titration.

Terence Featherstone, Ph.D., Biosciences Quality Control Manager
19-Jun-2007

Life Technologies Corporation, on behalf of its Invitrogen business, Molecular Probes® labeling and detection technologies, certifies on the data above that this is an accurate record of the analysis of the subject lot and that the data conform to the specifications in effect for this product at the time of analysis.
B.4.2 Certificate of Analysis for sPSL particles

Molecular Probes®

Certificate of Analysis

Catalog Number: S37486
Product Name: Sulfate latex, 8% w/v 0.8 μm
Appearance: suspension
Medium: de-ionized water
Lot Number: 1129648

Negatively charged surfactant-free polystyrene microspheres with sulfate functional groups on the surface.
Surface charge is pH independent. Stable at wide range pH. Surface is hydrophobic in nature.
STORE AT 2 - 8°C, DO NOT FREEZE

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<td>Coefficient of Variation of Diameter</td>
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<td>≤7 %</td>
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<td>Percent Solids w/v</td>
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<td>7.5 - 8.5 %</td>
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<td>Particle Number Per Milliliter of Latex</td>
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<td>Refractive Index of Polystyrene at 589 nm, 20°C</td>
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<td>Charge Content</td>
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<td>Charge Groups per Particle</td>
<td>5.6 x 10^5</td>
<td>n.a.</td>
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</table>

1. Determined from transmission electron microscopy.
2. Measured from conductometric titration.

Betty Wood

Betty Wood, Quality Assurance Manager
29-Feb-2012

Life Technologies Corporation, on behalf of its Invitrogen business, Molecular Probes® labeling and detection technologies, certifies on the date above that this is an accurate record of the analysis of the subject lot and that the data conform to the specifications in effect for this product at the time of analysis.
APPENDIX C: EXPERIMENTAL DATA

C.1 Approximation model aPSL sPSL system

| Sample name | a    | b    | a/b  | R    | |Ωmax| | |Ωmax|2 |
|-------------|------|------|------|------|------|------|------|------|
| APSP001     | 2.58E-05 | 1.19E-05 | 2.160804 | 2.84E-08 | 10.32 | 106.49 |
| APSP002     | 2.40E-05 | 8.80E-06 | 2.723864 | 2.55E-08 | 53.052677 | 2814.59 |
| APSP003     | 2.22E-05 | 9.10E-06 | 2.443956 | 2.40E-08 | 127.482698 | 16251.84 |
| APSP004     | 1.13E-05 | 6.01E-06 | 1.883527 | 1.28E-08 | 57.74 | 3334.13 |
| APSP005     | 1.81E-05 | 8.03E-06 | 1.31E-03 | 1.28E-08 | 127.482698 | 16251.84 |
| APSP006     | 1.14E-05 | 7.67E-06 | 1.491525 | 1.38E-08 | 47.2835642 | 2235.74 |

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## C.2 Sample Individual Angular Velocity Measurement for aPSL sPSL system

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Figure C-1. (A) Raw angle of rotation of a typical aPSL-sPSL aggregate. (B) Optical microscopy image of aggregate showing measurement of initial angle.
Figure C-2. Approximating aggregate to consist of two spheres of unequal radii to account for gravitational rotation.

\[
\begin{array}{l|l|l}
\text{Gravitational Rotation} & \text{pp-pf} & 55 \\
\hline
0 & \eta & 0.00089 \\
\hline
\text{a1} & 9.26E-06 & g \\
\hline
\text{a2} & 1.54E-05 & \\
\hline
L & & \\
\hline
\Omega g & -8.22E-01 & \text{rad/s} \\
-4.71E+01 & \text{deg/s} & \\
\end{array}
\]

C.3 Estimate of standard deviation in zeta potential distribution

Assume a flat plate which has been segregated into grids of squares which will be the patches. Each patch can have either of the two charges, -60 mV or +50 mV. Therefore, the standard deviation in zeta potential from one patch to the other is given by,

\[
\sigma^2 = \frac{\Sigma_1^N (x_i - x_0)^2}{N}
\]

Where N is the number of patches. For a 4x4 grid, N = 16. Substituting the values,

\[
\sigma^2 = 8 \left(\frac{55^2 + 55^2}{16}\right)
\]

Therefore,

\[
\frac{\sigma}{\sqrt{N}} = 13.75 \text{ mV}
\]
REFERENCES


59 (1878), 1981.


35 Liu et al., “Resonance Light Scattering Spectroscopy Study of Interaction Between Gold Colloid and Thiamazole and Its Analytical Application.”


55 Jacob N Israelachvili and Hakan Wennerstrom, “Hydration or Steric Forces Between Amphiphilic Surfaces ?,” *La* 6, no. 16 (1990): 873–876.


Aspects 368, no. 1–3 (September 2010): 84–90.


93 Ashavani Kumar et al., “Silver-nanoparticle-embedded Antimicrobial Paints Based on Vegetable Oil,”

95 M. M. Salpeter, L. Bachmann, and E. E. Salpeter, "RESOLUTION IN ELECTRON MICROSCOPE RADIOAUTOGRAPHY", Journal of Cell Biology, (1969), vol. 41 no. 1 1-20


97 Kebede Beshah et al., “Diffusion-Weighted PFGNMR Study of Molecular Level Interactions of Loops and Direct Bridges of HEURs on Latex Particles,” Macromolecules 46, no. 6 (March 26, 2013): 2216–2227.


101 Nikon Microscopyu – Numerical Aperture

http://www.microscopyu.com/articles/formulas/formulasna.html