ZETA POTENTIAL AND COLLOID CHEMISTRY
Eric Olson

ABSTRACT

The US Food and Drug Administration often requests the measurement of zeta potential; however, there is virtually no guidance offered in any of the regulatory pharmacopoeias. Guidelines are available in other standards, and there exists a wealth of information in texts and publications. Many of these are taken from non-pharmaceutical industries where zeta potential is routinely used as part of quality control criteria.

This discussion outlines current guidance to aid quality units with potential issues and questions; reviews the complex topics of colloid chemistry and zeta potential; and provides insight to chemists who may require zeta potential determination, but who may not be well versed in these topics.

Basic colloid principles are discussed including definitions, types of colloidal systems, and common properties. Types of colloidal particles, micelles, and surrounding layers are detailed. Colloidal stability is discussed. Electokinetic effects and underlying theories by which electrophoretic mobility is used to calculate zeta potential are detailed. Sample preparation, the importance of sample history, determination of zeta potential, and isoelectric point measurement are discussed.

INTRODUCTION

The determination of zeta potential is often required when a pharmaceutical formulation involves micelles or when the shelf life of a product that contains fine particles is in question. For the sake of this discussion, fine particles are defined as those with a mean diameter of 10µm or less. FDA often requests pharmaceutical manufacturers and formulators to provide zeta potential data.

Zeta potential is a frequently misunderstood phenomenon. Zeta potential is rarely taught in most collegiate curricula. There is no guidance offered in the United States Pharmacopoeia, National Formulary (USP/NF), the British Pharmacopoeia (BP), the European Pharmacopoeia (EP), the Japanese Pharmacopoeia (JP), or in current International Conference on Harmonization (ICH) guidelines on the definition, determination, or reporting of zeta potential. There was an American Society for Testing and Materials (ASTM) standard, D4187-82 in effect, but that standard was retired and not replaced (1). More recently, the International Organization for Standardization (ISO) published two draft standards related to zeta potential and prepared a third, which has not yet been released (2-4). In addition, the International Union of Pure and Applied Chemists (IUPAC) published some guidelines (5).
Zeta potential, per the IUPAC definition, is “The potential at the plane where slip with respect to bulk solution is postulated to occur is identified as the electrokinetic or zeta potential, \( \zeta \)” (5). In simplified terms, it is a determination of the charge between two particles that keeps them separated from one another. It is important to note zeta potential is the potential at the slip plane, not the charge on the surface of the particle as is often assumed. Furthermore, zeta potential is a colloidal determination that is typically performed on a liquid-liquid system or a solid-liquid system. It is not to be confused with the static charge or electrostatic potential developed by moving or processing a dry powder.

It is also important to distinguish between a solution and dispersion. A solution is defined as a system in which a solute is dissolved in a solvent. A colloidal system sometimes referred to as a dispersion, suspension, or sol, consists of a dispersed phase, which is distributed uniformly in a continuous phase.

There are two primary forces at work on colloidal systems: long range, weak, Van der Waals attractions and short range, stronger, electrostatic repulsions. It is the sum of the weak attractive and strong repulsive forces that are responsible for colloidal stability. Thus, the greater the magnitudes of the zeta potential, the more stable the colloidal dispersion. Because zeta potential is related to colloidal stability, it is often correlated to properties such as shelf life, material stability, and end-user mixing requirements. Though a zeta potential of high magnitude is often considered ideal, that is not always the case. In some applications (e.g., wastewater treatment) a low zeta potential is often desired. A low zeta potential can be established that will induce flocculation of the particles and aid in the water clarification process.

**METHOD VALIDATION**

Good manufacturing practice (GMP) method validations typically include specificity, linearity, range, accuracy, precision, and robustness. Specificity and linearity are not applicable to the determination of zeta potential. Range can be a difficult factor to assess because no single sample type can be used that will span the entire range of the instrument technique. There is no National Institute of Standards and Technology (NIST) traceable certified reference material for zeta potential to date, though several of the zeta potential instrument manufacturers offer a reference standard that is used for the purposes of determining accuracy and system suitability.

The electrokinetic effect known as electrophoretic mobility is normally measured and from that, the zeta potential is calculated. Per the draft ISO standard 13099-2, the accuracy is considered acceptable if the mean electrophoretic mobility value is within 10% of the published value (3). The repeatability of three measurements is considered acceptable if the coefficient of variation for the mean electrophoretic mobility values is less than 10%. The intermediate precision is considered acceptable if the coefficient of variation for the mean electrophoretic mobility value is less than 15%.
COLLOIDS

In order to discuss zeta potential in some detail, it is imperative to begin by defining the particles and systems to which zeta potential pertains. Zeta potential is measured in liquid-liquid or solid-liquid colloidal systems. A colloidal system consists of a dispersed phase that is distributed uniformly in a continuous phase. The dispersed phase contains particles (or micelles) that have a linear dimension between 1 nm and 1 um (6). Examples of various colloids are given in Table I.

Table I: Examples of colloidal systems.

<table>
<thead>
<tr>
<th>Dispersed Phase</th>
<th>Continuous Phase</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid</td>
<td>Gas</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Solid</td>
<td>Gas</td>
<td>Smoke</td>
</tr>
<tr>
<td>Gas</td>
<td>Liquid</td>
<td>Foam</td>
</tr>
<tr>
<td>Liquid</td>
<td>Liquid</td>
<td>Liquid Emulsion</td>
</tr>
<tr>
<td>Solid</td>
<td>Liquid</td>
<td>Sol or Suspension</td>
</tr>
<tr>
<td>Gas</td>
<td>Solid</td>
<td>Solid Foam</td>
</tr>
<tr>
<td>Liquid</td>
<td>Solid</td>
<td>Gel or Solid Emulsion</td>
</tr>
<tr>
<td>Solid</td>
<td>Solid</td>
<td>Alloy</td>
</tr>
</tbody>
</table>

Because of the linear size of the dispersed phase, colloidal particles have various properties in common. Given a colloidal particle is between 1 nm and 1 um, and the wavelength of visible light is between approximately 400 to 800 nm, they are both similar in dimension. Per Rayleigh scattering, the intensity of the scattered light is proportional to the frequency to the fourth power. Hence, blue light is scattered more efficiently than red light so colloidal dispersions often have a blue tint when illuminated with white light. In Figure 1, a Nd:YAG laser at a wavelength of 532 nm is shone from the right through a vial of filtered deionized water, which is adjacent to a vial containing water and a polystyrene colloidal particle at 500 nm. Notice the laser beam is not visible in the filtered water that is particle-free, but is clearly visible in the water with the 500 nm polystyrene particle. The beam is visible because of the dispersion of the green laser light at 532 nm by the polystyrene particles, which were nearly the same size at 500 nm. This effect is sometimes referred to as the Tyndall effect.
Due to the very small particle size of a colloidal particle, Brownian motion is sufficient to keep them suspended (7). At some point, the suspension of a particle by Brownian motion will be overcome by gravity and become subject to Stokes’ law (8). Stokes’ law is given as Equation 1, where $v$ is the settling velocity, $d$ is the particle diameter, $g$ is gravitational acceleration, $\Delta \rho$ is the difference in density between the particle and the continuous phase, and $\eta$ is the continuous phase viscosity.

$$v = \frac{d^2 g \Delta \rho}{18 \eta} \quad \text{[Equation 1]}$$

Another property that colloids have in common is a very high surface-to-volume ratio. As an example, consider two spheres, one with a radius = 0.250 $\mu$m and one with a radius = 100 $\mu$m. The resulting surface area and volumes are given in Table II. The exceptionally high surface area-to-volume ratio of the colloidal sphere illustrates the importance of surface chemistry in the colloidal domain. As one might expect, the behavior and stability of colloidal dispersions is dependent on the surface chemistry of the colloidal particles.

<table>
<thead>
<tr>
<th>Radius</th>
<th>Surface Area</th>
<th>Volume</th>
<th>Surface Area/Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.250 $\mu$m</td>
<td>$7.85 \times 10^{-1} \mu$m$^2$</td>
<td>$6.54 \times 10^{-2} \mu$m$^3$</td>
<td>12.0 $\mu$m$^2$/\mu$m$^3$</td>
</tr>
<tr>
<td>100 $\mu$m</td>
<td>$1.26 \times 10^5 \mu$m$^2$</td>
<td>$4.19 \times 10^6 \mu$m$^3$</td>
<td>0.03 $\mu$m$^2$/\mu$m$^3$</td>
</tr>
</tbody>
</table>
TYPES OF COLLOIDAL PARTICLES

Definitions and terminology associated with colloids are used in an inconsistent manner. Many texts define aggregation and agglomeration one way and others define them the opposite way. There were also a large number of texts that chose not to use the term “aggregate,” but term all particles larger than a primary particle an “agglomerate.” Terminology used in this discussion will follow the definitions in the ISO standard on sample preparation (9). It is important to know the type of colloidal particle for which zeta potential is to be measured to ensure the resulting zeta potential data are applicable to the system of interest.

**Primary Particle**

A primary particle is one that may be defined as the smallest unit or structure from which other structures are built. A primary particle may be a single crystal, a micelle, or even a small structure such as a polymeric sphere.

**Coalescence**

Certain primary particles may come together and coalesce. This generates a larger particle with a change in surface area compared to the sum of surface areas of the individual primary particles. A common example of coalescence, though not on the colloidal scale, is what happens with puddles of mercury. Two small puddles coalesce into a larger one with roughly the same shape, but with a decrease in total surface area.

**Ripening**

If the colloidal particle has some solubility in the continuous phase, a different phenomenon may occur. Small particles generally exhibit more surface strain, which leads to higher surface energy and an increase in solubility. Thus, the preferential solubility of the small particles will be greater than that compared to the larger particles. Over time, the population of small particles will dissolve into solution. Sometimes the dissolved material from the small particles will then deposit on the larger particles, making them even larger. This process is known as Ostwald ripening (10-12).

**Aggregation**

Primary particles may also undergo a process by which they come in close contact, stick to each other, and often form strong chemical bonds over a small area or point of contact. This process is called aggregation or in some references, coagulation. Aggregates of very small or few primary particles may still be in the colloidal size domain. Because of the bonds between the primary particles, they are most often quite difficult to separate by normal means. Note that the process of aggregation may or may not result in an appreciable difference in surface area. Whether it does is often dependent on the particle shape, the degree of particle-particle contact, and the extent of three-dimensional packing.
Agglomeration

The final type of colloidal particles to be mentioned is that of agglomeration, or in some references, flocculation. Primary particles or aggregates come in close contact and stick to each other by means of weak, long-range bonds such as hydrogen bonding by this mechanism. Agglomerates are often outside the colloidal size domain, but not always. Because the bonds are weak and typically long range, they are generally easy to break by normal processing means such as mixing, shaking, or sonication. Agglomeration does not generally proceed with an appreciable difference in surface area. Examples of the aforementioned particles and processes are illustrated in Figure 2.

![Diagram of colloidal particles showing primary particles, coalescence, Ostwald ripening, aggregation or coagulation, and agglomeration or flocculation.](image-url)

*Figure 2: Types of colloidal particles.*
MICELLES OR ASSOCIATION COLLOIDS

Sometimes in liquid-liquid systems, one component is more hydrophobic (water hating) than the other, which may be hydrophilic (water loving). If given enough time to equilibrate, the two components would separate into their own layers or phases. In order to stabilize a system like this, a surfactant is typically used.

Surfactants are a general class of molecules that have two ends: one that is hydrophobic and the other that is hydrophilic. The hydrophobic ends are often called “tail groups” and are typically nonpolar. These groups are commonly comprised of long covalently-bonded alkyl groups such as laurate (C₁₂), myristate (C₁₄), palmate (C₁₆), stearate (C₁₈), unsaturated alkyl groups such as linolate (C₁₈:2) and oleate (C₁₈:1); or contain aromatic functionality such as C₁₄H₂₂O(C₂H₄O)₁₀H (octyl phenol ethoxylate). Hydrophilic ends or “head groups” are typically polar, ionic, and are often comprised of chemical groups such as PO₄³⁻ and SO₄²⁻.

When added to a typical liquid in very low concentrations, the surfactant will form a monolayer on the surface of the liquid-gas interface. As the concentration of surfactant increases, a point will be reached, called the critical micelle concentration (CMC), at which time the surfactant molecules will self-assemble into micelles as shown in Figure 3. Micelles are abundantly found in nature and are of great importance in the pharmaceutical field as drug-delivery vehicles (13). It is the micelle in a liquid-liquid colloidal system that has a charge and that for which zeta potential may be measured. In Figure 3, the head groups are illustrated as red spheres and the tail groups are illustrated as black alkyl chains. This process is driven by thermodynamics and is nearly spontaneous.

![Figure 3: Cross-sectional depiction of a typical micelle.](image-url)
The CMC is a complex property and is dependent on a variety of factors (6). Some of these factors include the chain length of the surfactant, the concentration of electrolytes in the dispersion, the size, and polarizability of the electrolytes in the dispersion, temperature, etc. The ability to measure the CMC of a given system is a common request in this field, but is outside the scope of this article.

This paper generally focuses on the most common type of micelle, the spherical micelle. However, other shapes such as cylinders, flexible bilayers, planar bilayers, and inverted micelles do exist. The shape of the micelle is generally dependent on the surfactant type (single-chain vs. double-chain), the size of the hydrophilic head group, the type of head group, the dispersion electrolyte concentration, pH, and the degree of surfactant branching (14).

ANATOMY OF A COLLOIDAL PARTICLE

A particle or micelle in a colloidal system is a dynamic entity and is not isolated or static. As will be discussed, particles or micelles are surrounded by multiple layers of solvated ions and counter ions that are in constant motion, even at a point of stable equilibrium (15-21). If these layers of ions and fluid are considered fixed distances from the solid particle surface, the colloidal particle can be idealized more as a two-dimensional set of concentric circles. At the center of these concentric circles is the particle or micelle itself.

Particle Surface and Electrophoretic Mobility

The majority of commercial instrumentation for the determination of zeta potential utilizes the principle of electrophoretic mobility, which is defined as the movement of charged colloidal particles under the influence of an external electric field. Thus, in order to measure the electrophoretic mobility and subsequent zeta potential, the colloidal particle or micelle in question must have a charge.

There are a number of mechanisms by which a solid colloidal particle may acquire a surface charge, some of which include preferential adsorption of ions, dissociation of surface groups, and differential loss of ions. Similarly, the surface charge of a micelle is dependent on the number of exposed functional groups on the surface, the types of functional groups, the packing density, and other factors. The plurality of mechanisms underscores the fact that the charge on the surface of the colloidal particle or micelle is not only a function of the exposed chemical groups at the surface, but also the surrounding ions from the environment, the temperature, diffusion rate of the ions, possible steric hindrance, or shielding effects.
The fact the charge on the surface of the particle is a function of the exposed chemical or functional groups is often overlooked. There have been many instances where a particular active pharmaceutical ingredient (API) behaved favorably in a formulation (i.e., having acceptable stability). Once a change was made in the manufacturing of the API, the API no longer functioned in the formulation properly (i.e., leading to poor stability). Most typical quality control tests are not designed to determine what has happened to the API, only that the formulation is no longer stable. A long investigation may ensue that eventually leads back to the change, often in milling or recrystallization of the API. What has been overlooked is that by milling, fresh surfaces may be exposed that contain different crystal faces, functional groups, and consequently, different surface charges. Likewise, recrystallization from a different solvent or with alternate conditions may also lead to different exposed crystal faces and functional groups.

**Stern Layer and Diffuse Layer**

Once a charged colloidal particle is dispersed in a liquid continuous phase or a micelle is formed, there will exist a layer of tightly bound ions that are of opposite charge to those of the surface; this layer is called the Stern layer. Beyond the Stern layer is another layer called the diffuse layer. The diffuse layer contains loosely bound ions, mostly which are opposite in charge to those found in the Stern layer. See Figure 4 for a representation of a typical colloidal particle.

![Figure 4: Typical colloidal particle.](image)
Shear Plane

When a dispersed colloidal particle is exposed to an electric field, the particle, closely bound Stern layer, and a portion of the loosely-bound diffuse layer will be attracted (or repelled) to or from the poles of the field depending on the charge of the particle. Because the diffuse layer is only loosely bound, a portion of the layer will move with the particle and a portion will not, creating a plane of shear. This is called the shear plane or is sometimes referred to as the slip plane. It is at the shear plane where the electrophoretic mobility is measured and the zeta potential is determined. The Stern layer and portion of the loosely bound diffuse layer is sometimes referred to as the electrical double layer. The thickness of the double layer is called the Debye length, and is given the symbol, $\frac{1}{\kappa}$.

COLLOIDAL STABILITY

The stability and properties of colloidal systems have been studied for decades by a variety of individuals (22-40). Colloids have also been modeled using a variety of approaches and for a variety of geometric configurations including flat plates, cylinders, and spheres. Most of the mathematics and intricacies are beyond the scope of this article, but some of the concepts require further explanation.

At the core of understanding colloidal stability are two opposing forces: Van der Waals attractions and electrostatic repulsion. Van der Waals forces arise from atomic and molecular level interactions due to induced and permanent dipoles in the system. They include Keesom interactions (i.e., from permanent dipole/permanent dipole interactions), Debye interactions (i.e., from permanent dipole/induced dipole interactions), and London interactions (i.e., from induced dipole/induced dipole interactions). They are always attractive (negative in sign), are relatively weak, and are relatively long ranged.

The electrostatic repulsive forces are generally Coulombic in origin, are relatively strong, and are relatively short ranged. In this case, they are also repulsive (i.e., positive in sign). Much more detail on all the forces can be found in Israelachvili (1991) (14).

In order for a colloidal system to be stable, the repulsive forces between the particles must be greater than the attractive forces. A typical potential energy plot vs. interparticle distance is given in Figure 5.
Figure 5 indicates a few features of note. In order from the right (greatest interparticle distance), it is shown that at infinite distance, the potential between the particles approaches zero. As the particles approach each other and the interparticle distance decreases, there exists a potential secondary minimum ($2^\circ$ min.) in certain systems. Particles that enter the secondary minimum are weakly bound and are said to be agglomerated or flocculated. The floccs, if present, are very loosely bound with an open structure and low particle packing fraction. Flocculation is generally reversible with a small amount of required energy. Note the secondary minimum is often quite broad and shallow.

As the particles approach closer, the repulsive forces manifest themselves in an energetic barrier or activation energy, $E_a$. If this barrier is large enough, the particles will remain in or near their secondary energetic minimum due to kinetics. However, if the particles come close enough to one another to overcome the repulsive barrier, they will descend into the thermodynamic primary minimum ($1^\circ$ min.). Particles that enter the primary minimum are strongly bound and are said to be aggregated. The aggregates are tightly bound with a dense structure and high particle packing fraction. Aggregation is normally considered an irreversible process.

At the other extreme end of the plot, where the interparticle distance approaches zero, the energetic potential drastically increases as the particles begin to experience nuclear repulsion.
IMPORTANT FACTORS IN COLLOIDAL STABILITY

Given the plot in Figure 5, one might expect the most efficient manner to develop a highly stable colloidal system would be to increase the energetic barrier, $E_a$, which prevents the particles from experiencing irreversible aggregation at the primary energetic minimum. A list of significant factors that affect colloidal stability can be derived through examination of the various theories. This list of factors generally includes pH, ionic strength or conductivity, and temperature.

**Temperature**

Of these, temperature may be the easiest to conceptualize. As is the case with all chemical reactions, the degree to which a reaction proceeds as a function of time is dependent on thermodynamics and kinetics (41-43). In general, as the temperature of a reaction increases, so does the available energy, $kT$, and the subsequent kinetic rate. Thus, one might expect the relative proportion of particles that are able to overcome the energetic barrier to increase with an increase in temperature. Of course, temperature also affects the Brownian motion of the particle and the dynamic viscosity of the liquid continuous phase (7, 44). This is why colloidal measurements are often conducted at very controlled temperatures.

**pH**

Another very important factor is pH. As previously mentioned, one of the major sources of surface charge is the dissociation of surface groups. An example of this mechanism would be protonation or deprotonation of a particular functional group at the particle surface. Like a zwitterion, many surfaces can adopt a negative or positive charge, depending on the pH. This, in turn, can lead to a negative or positive zeta potential, the magnitude of which may be a function of pH.

**Debye Length and Ionic Strength**

Finally, $1/\kappa$, called the Debye length, approximates the double layer thickness, where $N_A$ = Avogadro’s number, $z$ = ionic valence, $M$ = concentration of each ion in the system, $\varepsilon$ = dielectric constant, $k_B$ = Boltzmann’s constant, and $T$ = temperature.

$$\kappa = \sqrt{\frac{2000e^2N_A\sum z_i^2M_i}{\varepsilon k_B T}}$$

[Equation 2]
As shown by Equation 2, as the ionic strength increases, the Debye length decreases. This effectively increases the shielding effect of the double layer, thus decreasing the measured zeta potential. In fact, at a given ionic strength called the critical coagulation concentration, most colloids can be forced to undergo catastrophic instability, which is sometimes called “salting out” of the system.

Another interesting property of colloids comes from the equation for the ionic strength, $I$.

$$I = \sum_i z_i^2 M_i$$  \hspace{1cm} [Equation 3]

As shown, the ionic strength is a factor of the ionic valence squared. Thus, monovalent ions such as Na\(^{+1}\), K\(^{+1}\), Cl\(^{-1}\), and Br\(^{-1}\) contribute to the ionic strength as a function of their respective concentrations. However, even trace levels of polyvalent ions such as Mg\(^{+2}\), Ca\(^{+2}\), and Al\(^{+3}\) can increase the ionic strength of the system disproportionately and cause colloidal instability. It is for this reason why many wastewater treatment facilities use FeCl\(_3\) as a flocculant (45).

ZETA POTENTIAL AND ELECTROPHORETIC MOBILITY

Electrokinetic effects may be defined as those phenomena involving tangential fluid motion adjacent to a charged surface. There are a few electrokinetic effects often considered in colloidal systems: electrophoresis, electro-osmosis, streaming potential, sedimentation potential, and electrokinetic sonic amplitude. The majority of commercial instrumentation for the determination of zeta potential utilizes the principle of electrophoresis.

Electrophoresis

Electrophoresis is defined as the movement of charged colloidal particles under the influence of an external electric field. The electrophoretic velocity, $v_e$ (m s\(^{-1}\)), is the velocity during electrophoresis. The electrophoretic mobility, $u_e$ (m\(^2\) V\(^{-1}\) s\(^{-1}\)), is the magnitude of the velocity divided by the magnitude of the electric field strength.

Once the electrophoretic mobility, $u_e$, has been measured, several mathematical equations may be used to relate it to the zeta potential, $\zeta$. The proper equation is determined by first evaluating $kr$, where $1/\kappa$ is the Debye length and $r$ is radius of the particle or micelle. Once $kr$ is determined, the initial zeta potential is calculated. Using the initial zeta potential determination, further refinements, or alternate equations may be used as shown in the flowchart of Figure 6.
If $\kappa r$ is large, say $>20$, then the Debye length is generally small. This is common in many aqueous systems. If the absolute value of the initial measured zeta potential, $|\xi|$, is $\leq 50$ mV, then it can be assumed the diffuse layer is not conductive. In this case, the equation is called the Helmholtz-Smoluchowski equation [Equation 4]. $\varepsilon$ is the dielectric constant and $\eta$ is the dynamic viscosity of the system.

$$u_e = \frac{\varepsilon \xi}{\eta}$$  \hspace{1cm} [Equation 4]

Note the dependence of the electrophoretic mobility on the dielectric constant and viscosity. $u_e$ is directly proportional to $\varepsilon$, so in aqueous systems where $\varepsilon$ is very high (80.1 @ 20°C), $u_e$ is typically very high. Conversely, in organic systems such as methanol where $\varepsilon$ is very low (30 @ 20°C), $u_e$ is typically low. Likewise, $u_e$ is indirectly proportional to $\eta$, so in aqueous systems where $\eta$ is very low (1.002 cP @ 20°C), $u_e$ is typically very high and vice versa. It is for this reason that many commercial instruments have difficulty measuring zeta potential on systems whose dynamic viscosity is greater than about 10 to 20 cP.

If the absolute value of the initial measured zeta potential is $\geq 50$ mV, then the diffuse layer cannot be assumed to be non-conductive and should be taken into account by using the O’Brien-White equation [Equation 5] (46).
z is the electrolyte valence, ζ is the zeta potential, k is Boltzmann’s constant, T is the absolute temperature, D is the ionic diffusion rate, r is the radius of particle or micelle, η is the viscosity, 1/κ is the Debye length, and ε is the diluent dielectric constant.

If κr is small, say <1, then the Debye length is generally large. This is typical of many non-aqueous systems. It may also hold true for aqueous systems with very low ionic strength. In this case, the Debye-Hückel equation [Equation 6] should be used.

$$u_e = \frac{2 \zeta \epsilon}{3 \eta}$$

[Equation 6]

If κr is >1 but <20, then the Debye length is a moderate size, which is perhaps the most common scenario for aqueous systems that have also have an average ionic strength. If the absolute value of the initial measured zeta potential is ≤ 50 mV, then it can be assumed the diffuse layer is not conductive. In this case, the preferred equation is called Henry’s equation [Equation 7]. Note Henry’s equation is a generalized equation such that if κr = α, then as α approaches 0, f (α) approaches 1, and Henry’s equation reduces to the Debye-Hückel equation. Likewise, as α approaches ∞, f (α) approaches 3/2, and Henry’s equation reduces to the Helmholtz-Smoluchowski equation.

$$u_e = \frac{2 \zeta \epsilon}{3 \eta} f(\alpha)$$

[Equation 7]

If the absolute value of the initial measured zeta potential is ≥ 50 mV, then the diffuse layer cannot be assumed to be non-conductive and should be taken into account by using Oshima’s equation [Equation 8] (47). In Equation 7, K_p is a factor to compensate for the conductivity of the diffuse layer. As can be seen, Henry’s equation and Oshima’s equation are very similar and in the limit that the diffuse layer becomes non-conductive, Oshima’s equation does reduce to Henry’s equation.

$$u_e = \frac{2 \zeta \epsilon}{3 \eta} f(\alpha, K_p)$$

[Equation 8]
SAMPLE PREPARATION

A frequently asked question in this field is, “what is the zeta potential of my dry powder sample?”

This is a very difficult question to answer because by definition, a dry powder sample does not have a zeta potential. The dry powder sample cannot establish an electrical double layer until it is dispersed in a continuous liquid phase; thus, there is no electrophoretic mobility to measure and no zeta potential to determine. This raises the issue of sample preparation.

As previously mentioned, the stability of a colloidal system is dependent on a number of factors. Some of these include pH, ionic strength, and temperature. These must be closely monitored when making a colloidal dispersion. One thing that is often ignored is the “history” of the dispersion. In many systems, the colloid “remembers” its history. Though the explanation and fields of rheology and fluid dynamics are beyond the scope of this paper, it is important to note the type, amount, and duration of sheer applied to the colloidal system. For instance, a sonic bath may not yield the same product as a sonic probe. Likewise, a small-scale blender batch may not yield the same product as a full-scale industrial mixer. Not only are the sheer profiles different, but the time of application and in many cases, the resulting temperature levels, are different if not controlled.

It is often important to note the concentration or volume fraction of the particles during the dispersion process. The process history (i.e., if the dispersion has undergone filtration, centrifugation, or dilution) is also important. Some colloidal systems are sensitive to ionic radii. NaCl may not behave the same as KCl in certain systems because of the mismatched difference between the ionic radii of Na\(^+\) (1.16Å) and K\(^+\) (1.52Å) and that of Cl\(^-\) (1.67Å) (48). The age of the dispersion may also make a difference in the zeta potential determination, especially if the colloidal particle experiences ripening. Storage conditions, especially temperature conditions, may drastically affect a colloid. All these may have an effect on the resulting zeta potential determination and colloidal stability.

When dealing with colloidal systems, it is often necessary to dilute them in order to obtain particle size or zeta potential determinations. This is because many instruments that measure zeta potential do so through use of electrophoretic light scattering. Thus, some colloidal systems have to be diluted in order to minimize multiple particle light scattering events. When a colloidal system is ultracentrifuged to separate the particles from the continuous phase, the supernatant still contains the same concentration and type of ions as the parent dispersion. The supernatant, called the “mother liquor”, may be used as the preferred diluent for the parent dispersion. In this way, the volume fraction of solids may be reduced without changing the electrolyte background.

Consideration of all the above factors needs to occur prior to sample preparation when performing zeta potential determination. This is especially true if comparison between data sets is the end goal.
ZETA POTENTIAL DETERMINATION

When measuring zeta potential, a few limitations must be considered. Zeta potential cannot be measured on a particle that is anchored to a substrate or locked in place by a solid continuous phase. The colloidal particle must be free to move. In addition, the particle must have a large enough charge and small enough size to move at a detectable rate when exposed to an electric potential. The upper limit on mean particle diameter for which zeta potential may be measured is dependent on several factors, but in general, is between 1 to 10 μm.

There are several ways to measure zeta potential. Modern instrumentation primarily utilizes two principles: electrophoretic light scattering and electrokinetic sonic amplitude (ESA). Electrophoretic light scattering can be further divided into those methods that measure the resulting change in frequency and those methods that measure the resulting change in phase.

Electrophoretic Light Scattering

When measuring zeta potential by electrophoretic light scattering, a dispersion is placed in a cell that has a pair of electrodes at a fixed and known distance apart. A potential is then applied across the electrodes. The colloidal particles will migrate to or away from the electrodes, and the direction and velocity will depend on the charge of the particle and its environment. When a coherent incident light such as that from a laser strikes the particle and is scattered, there is a change in the frequency and phase of the scattered light. If the change in frequency is utilized, then a Fourier transform of the data is utilized to convert the data from frequency domain to time domain. Once the data are expressed as a function of time, the velocity, electrophoretic mobility, and subsequent zeta potential can be calculated. If the change in phase is utilized, the determination is called phase analysis light scattering (PALS). In the case of PALS, the data are expressed as a function of phase, which is already a function of time. Thus, a Fourier transform is not necessary and the velocity, electrophoretic mobility, and subsequent zeta potential can be calculated with greater ease, less computational requirement, and with greater sensitivity.

Electrokinetic Sonic Amplitude

When utilizing ESA, a dispersion is placed in a cell that has a pair of electrodes at a fixed and known distance apart. An alternating current is then applied across the electrodes. When an alternating current propagates through a colloidal dispersion, it causes the particles to vibrate in a way that depends on their size and on their zeta potential at the frequency of the applied field. If there is a density difference between the particles and the liquid, this motion will generate an acoustic wave of the same frequency as the applied electric field (49-53). The sound wave is then detected by a very sensitive piezoelectric detector.
The ESA amplitude can then be used to calculate the electrophoretic mobility and zeta potential in a similar manner as that for the other electrokinetic effects using Equation 9. \( ESA \) is the electrokinetic sonic amplitude, \( \eta \) is the viscosity, \( \varepsilon \) is the dielectric constant, \( \phi \) is the volume fraction, \( \Delta \rho \) is the difference in density between the particle and the diluent, \( c \) is the speed of sound in the diluent, and \( G(a)^{-1} \) is a factor to take into account the inertial forces of the particles at high frequency.

\[
\zeta = \frac{ESA \eta}{\varepsilon \phi \Delta \rho c} G(a)^{-1}
\]

[Equation 9]

**ISOELECTRIC POINT DETERMINATION**

A common measurement that is often associated with zeta potential determination is the isoelectric point determination. The definition of the isoelectric point is the pH at which the electrophoretic mobility is zero. As previously mentioned, a colloidal dispersion is considered stable if the magnitude of the zeta potential is large, and this is typically desired. However, there are some cases where it is desired for the dispersion to be unstable. A colloidal system is generally least stable at its isoelectric point.

In order to determine the isoelectric point, the most common method is to measure the zeta potential of a colloidal system over a wide range of pH values. The pH values are normally adjusted by the addition of an acid or base in a typical pH titration manner. For example, as the pH of a particle with \(-\text{OH}\) surface groups is decreased, the degree of protonization will increase, yielding a net positive charge on the surface and a positive zeta potential. The opposite is also true. As the pH is increased, the degree of deprotonization will increase, yielding a net negative charge on the surface and a negative zeta potential. Remember the zeta potential is not the surface potential, but rather the potential measured at the shear plane, which is a function of the surface charge as well as all the other factors mentioned earlier. The isoelectric point, like the zeta potential, is not only dependent on the surface charge and other factors, but is also sometimes useful in determining purity of the colloidal particle as well as giving insight into the history of the particle. An isoelectric point titration for a typical colloidal system is shown (as the red data) in Figure 7.
Figure 7: Zeta potential titration of a common colloid before and after heat treatment.

Like all zeta potential determinations, the isoelectric point determination is sensitive to the history of the colloidal system. A specific example has been observed due to the processing temperature of a colloidal sample. In this example, the sample was produced in a pyrogenic process and calcined for a given time at a given temperature. The sample was then analyzed and the data are presented in Table III. Afterwards, the same batch of material was further heat treated under a nitrogen blanket, the surface annealed, and several –OH groups were lost in a condensation mechanism. This heat-treated sample was then analyzed and the data are presented in Table III. The isoelectric point titration for the heat-treated material is shown (as the blue data) in Figure 7. Clearly, the two colloidal particles were different because of their temperature histories and one would expect them to behave quite differently in a variety of applications.

Table III: Isoelectric point of pre- and post-heat treated material.

<table>
<thead>
<tr>
<th></th>
<th>B.E.T. Surface Area (m²/g)</th>
<th>Mean Diameter (nm)</th>
<th>Zeta Potential @ pH 10 (mV)</th>
<th>Isoelectric Point (pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As received</td>
<td>200</td>
<td>200</td>
<td>-45</td>
<td>2.0</td>
</tr>
<tr>
<td>Post heat treatment</td>
<td>70</td>
<td>200</td>
<td>-15</td>
<td>4.5</td>
</tr>
</tbody>
</table>
The process of properly titrating a system is not intuitive to most. A common mistake is to disperse a colloid at a given pH such as at pH 7. The pH of the dispersion is then decreased in increments to perhaps pH 1 as the zeta potential is measured. Then, the chemist erroneously titrates the dispersion up to pH 10, while taking zeta potential determinations. There are at least two striking errors in this methodology: the history of the system is ignored, and the effect on the zeta potential is not only due to the pH of the system, but is also due to the increase in ionic strength.

In the case of the first error, if the effect of conductivity is ignored, there is still the effect of the change in pH on the surface chemistry of the particle. As the pH decreases, this may affect the solubility of the surface. It may also affect the surface energy and distribution of functional groups, similar to how temperature and annealing affected the surface in the previous example. These changes to the surface are often irreversible. In fact, it is not uncommon to titrate a system down in pH while taking zeta potential determinations, then as the chemist titrates the pH back up, the zeta potential determinations do not agree with the initial determinations due to the irreversible changes on the particle surface. It is usually better to split the original dispersion then use one-half to titrate down and the other half to titrate up. For an example of this type of titration error, see Figure 8. In Figure 8, the red data represent the first part of the titration when the pH is decreased. The blue data represent the second part of the titration when the pH is increased. Note the hysteresis between the two data sets.

![Figure 8: Zeta potential titration of a colloidal system as it is commonly (and erroneously) performed.](image-url)
In the case of the second error, that of the differences in conductivity, there are two typical ways to overcome the error. One way is to disperse the sample in a continuous medium that has a relatively high ionic strength such as 0.1 M KCl. In this way, the change in conductivity due to the increase in ions from the acid or base is overwhelmed by the ionic strength of the diluent. This is definitely the easiest way to overcome the error, but due to the dependency of the zeta potential determination on conductivity, it may not be the most accurate.

An alternate method to overcome the conductivity error is to proceed as follows:

1. Using an aliquot of the prepared dispersion, adjust the pH to the maximum level required (i.e., pH 2 or pH 10).
2. Measure the conductivity at both ends of the pH range and determine which pH level yields the dispersion with the highest conductivity.
3. Using a fresh aliquot of the prepared dispersion, titrate the dispersion either up or down with base or acid, respectively. Concurrently, perform a conductivity titration by adding a dilute spectator ion such as KCl to the dispersion. The endpoint of each conductivity titration should be the highest conductivity measured in step 2.
4. Using another fresh aliquot of the prepared dispersion, titrate the dispersion the opposite pH direction with base or acid. Concurrently, perform a conductivity titration by adding a dilute spectator ion such as KCl to the dispersion. The endpoint of each conductivity titration should be the highest conductivity measured in step 2.

This methodology separates the history of increasing pH from that of decreasing pH. It also compensates for the change in conductivity due to pH titration, which effectively deconvolves the effects of pH from conductivity on the isoelectric point.
CONCLUSIONS

When requesting or measuring zeta potential, there are many factors that should or must be taken into consideration, some of which include the following:

- pH. Colloidal stability and the isoelectric point must be considered.
- Conductivity or ionic strength. Do not exceed the critical coagulation concentration (unless desired).
- Temperature. Excess temperature in either direction can destabilize a suspension.
- Particle size. The particles or micelles must be free to move and need to be small enough that they have a measureable electrophoretic mobility.
- Solubility (or lack thereof). Ensure the sample is a dispersion, not a solution.
- Viscosity of the diluents. Excessive viscosity can attenuate the electrophoretic mobility.
- Debye length and initial zeta potential. These are used to determine the proper equation to calculate zeta potential from electrophoretic mobility.
- History. This includes shear history, storage conditions, dilution, centrifugation, and filtration.

There is clearly a need for regulatory guidance in the international pharmacopeias. In the interim, there are draft ISO standards, textbooks, and numerous research papers that can be used and referenced.
REFERENCES

24. Chapman, S., Philos. Trans. R. Soc. London, Series A, Containing Papers of a Mathematical or Physical Character, 211, 1912.