Particle size measurement

1.
What is a particle?

Let’s start with the basics

We can define a particle as a discrete sub-portion of a substance, with physical dimensions ranging from subnanometers to several millimeters.

What is the size?

- 3D object (tridimensional);
- 50 x 30 x 30 (hxLxW);
- We need 3 numbers to describe the object's size.

2.
Importance of Particle Size

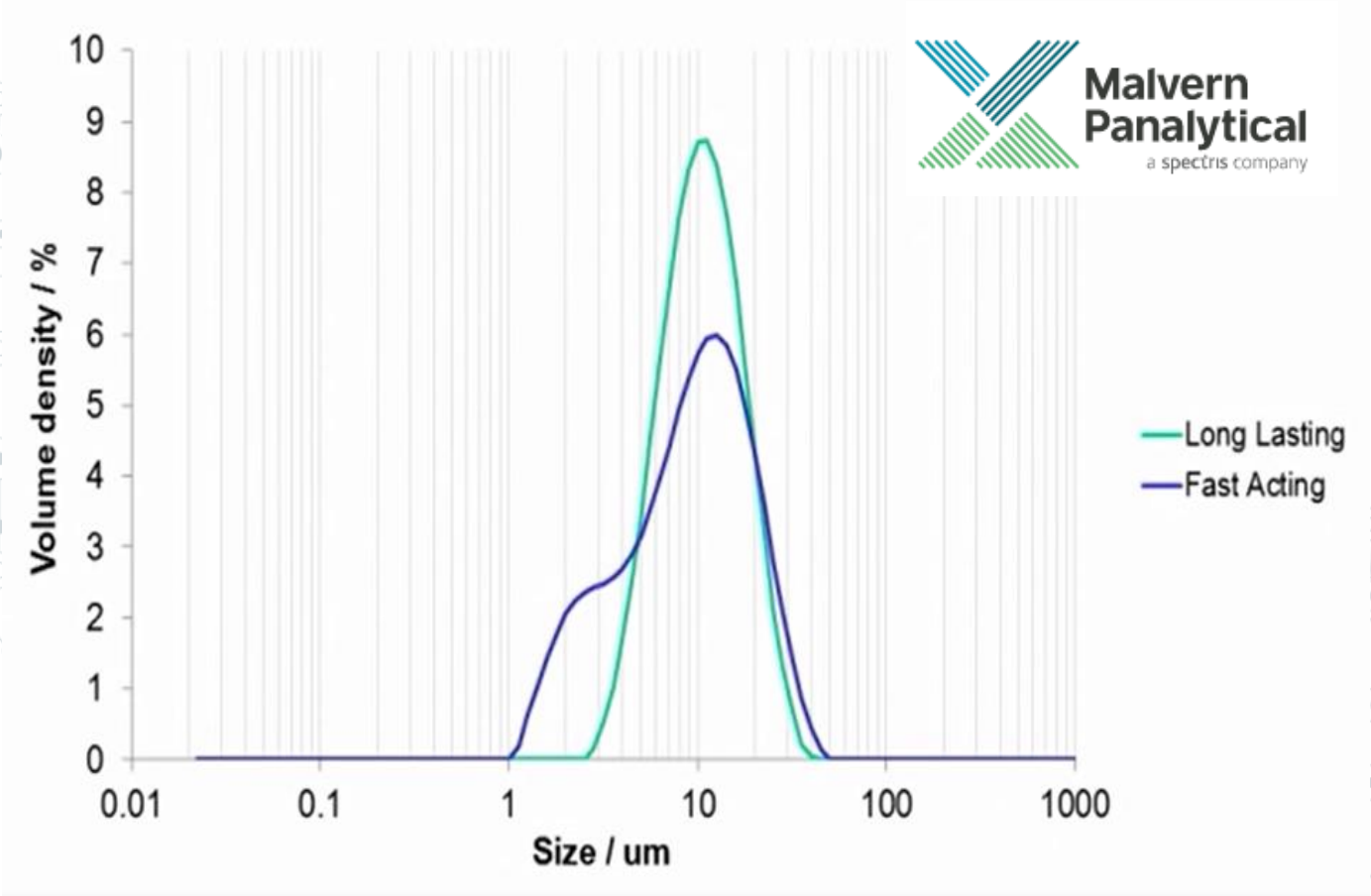
Why do we measure particle size?

To predict product performance:

- Dissolution
- Content uniformity
- Flowability of the raw material during manufacturing
- Suspension/sedimentation -> stability
- Change in taste/flavor
- Color alteration (paints)
- Material deposition (targeting/adhesiveness)

Process production controlling:

- Powder mixing
- Grinding/Milling
- Compression
- Nanoparticles development


Malvern Panalytical
a spectris company
Volume density / %
10
9
8
7
6
5
4
3
2
1
0
0.01
0.1
1
10
100
1000
Size / um
Long Lasting
Fast Acting

3.
Brownian motion

Brownian motion

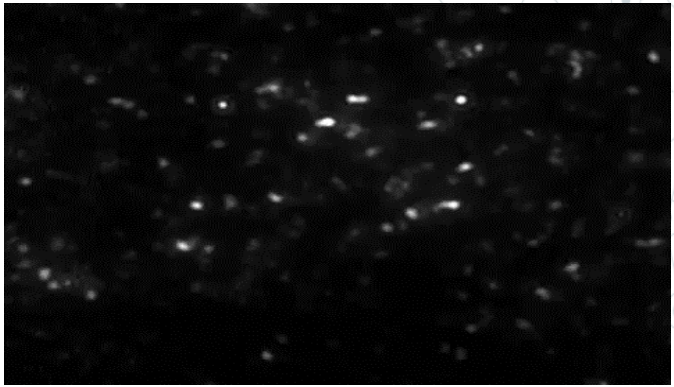
Brownian motion is the term used to describe the irregular movement of pollen suspended in water, observed by the botanist Robert Brown in 1828.

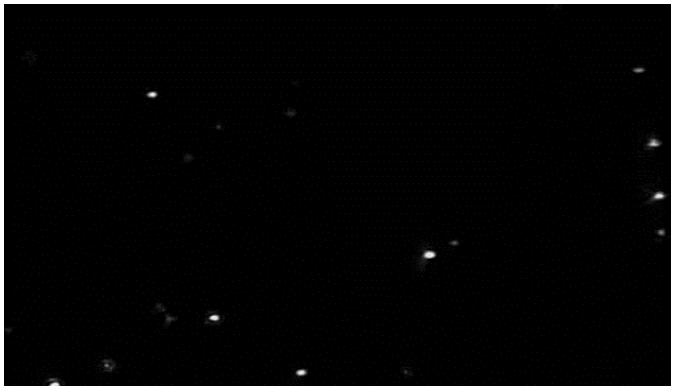
KARATZAS, Ioannis, et al. *Brownian motion and stochastic calculus*. Springer Science & Business Media, 1991.

Brownian motion

Definition:

Brownian motion is the random movement of particles due to the bombardment by solvent molecules surrounding them. The larger the particle or molecule, the slower the Brownian motion will be.





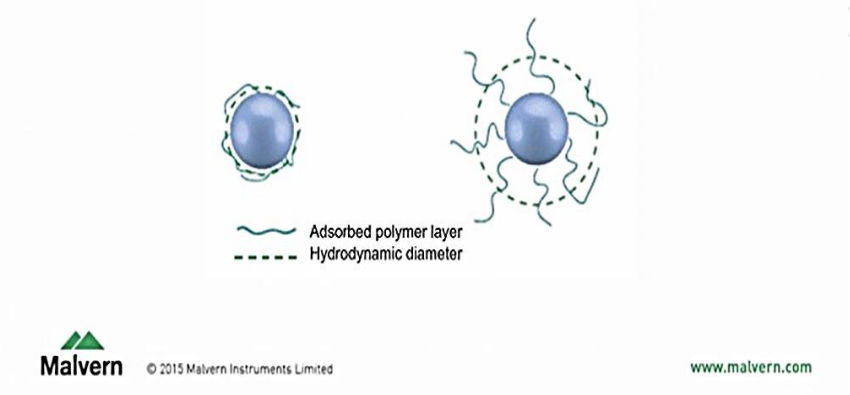
https://www.malvernpanalytical.com/en/learn/knowledge-center/technical-notes/tn101104dynamiclightscatteringintroduction

Brownian motion

Stokes-Einstein equation:

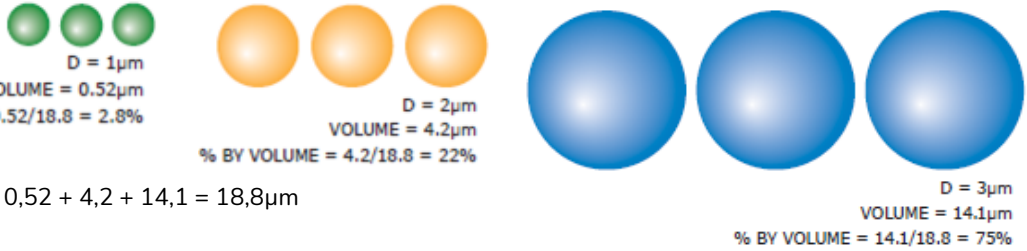
$$d(H) = \frac{kT}{3\pi\eta D}$$

d(H) = hydrodynamic diameter
D = translational diffusion coefficient
k = Boltzmann constant
T = absolute temperature
η = viscosity of the solvent



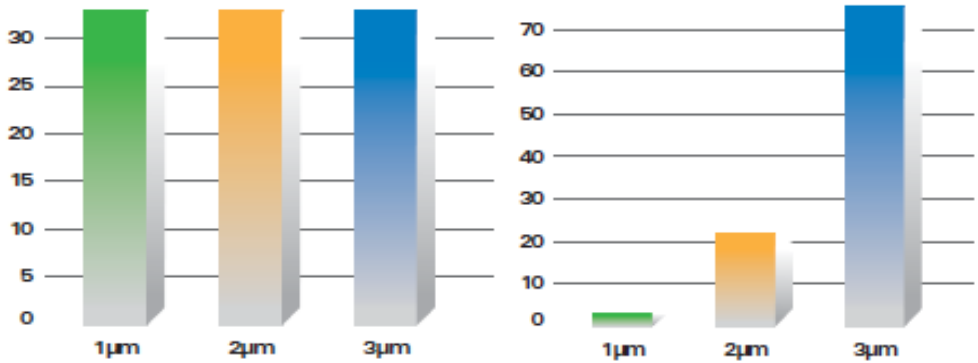
4.
Interpreting the results

Volume vs. Number distribution.



Total volume: 0,52 + 4,2 + 14,1 = 18,8µm

The graph on the left represents the number distribution, while the graph on the right represents the volume distribution.

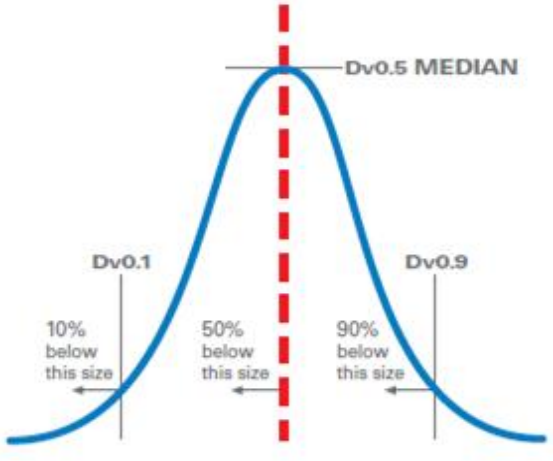


Volume vs. Number distribution. (Adapted from HORIBA INSTRUMENTS INC., 2016)

Results interpretation

The presentation of the results in a single number is not recommended. Several parameters are analyzed by the laser diffraction instrument; therefore, multiple numbers should be shown in the results. This way, the particle size distribution amplitude will be better interpreted:

- D90
- D10
- D50
- D4.3
- D3.2



Values on the X-axis for D10, D50, and D90. (Adapted from HORIBA INSTRUMENTS INC., 2016)

Results interpretation

In addition to the D10, D50, and D90 values, range calculations and the D4.3 value can be used.

- The range calculation (span calculation) describes the distribution amplitude of the found values.

$$Span = \frac{D_{v0,9} - D_{v0,1}}{D_{v0,5}}$$

- The value of the mean volume diameter can be expressed as D4.3. In the instruments, this value is simply called the mean since the results are given as volume distribution. The D4.3 value is converted to a surface value, meaning the particles are considered as if they were planar. This value is known as D3.2 or the surface mean.

$$D[4,3] = \frac{\sum_1^n D_{iVi}^4}{\sum_1^n D_{iVi}^3} \qquad D[3,2] = \frac{\sum_1^n D_{iVi}^3}{\sum_1^n D_{iVi}^2}$$

- All calculations and analyses should follow the ISO 13320 standard (Particle size analysis - Laser diffraction methods).

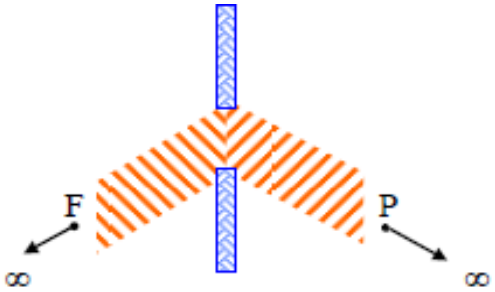
5.
Optical models

Fraunhofer approximation

This approximation provides good data for large particles and is not recommended for particles smaller than 50µm or for transparent particles.

$$\psi(x,y,z) \cong \psi_0 \frac{\lambda}{z}\frac{1}{A}\int x_0 \int y_0 \, \tilde{g}(x_0,y_0) exp\left[i2\pi\left(\frac{xx_0}{\lambda_z}+\frac{yy_0}{\lambda_z}\right)\right] dx_0 dy_0$$

It assumes that the particles are spherical, opaque, scatter light equally for narrow and wide angles, and interact with light differently from the surrounding medium



Fraunhofer Diffraction. (Adapted from ZILIO, 2009)

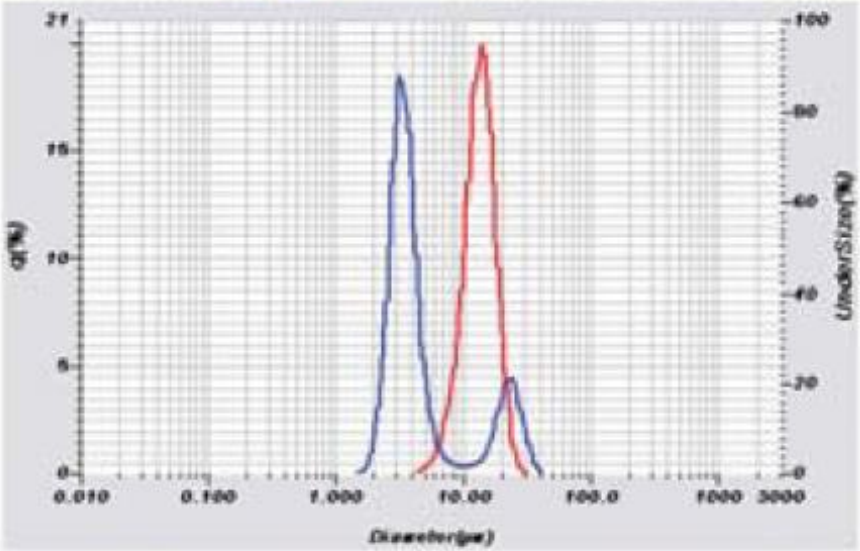
Mie theory

Mie diffraction model started to be used as it overcomes these limitations. This solution includes sensitivity to smaller particles, meaning larger angles and greater variation in opacity, such as light absorption.

Mie theory describes the spectrum of extinction, which is the absorption and scattering of light by particles under the following conditions:

1. The distance between particles must be greater than the wavelength of the incident light beam so that scattering in one particle does not affect the others in the medium.
2. The size of the particles must be smaller than the wavelength of the incident light.
3. The dielectric constant of the medium must be known.

Data Name	Graph Type	Refractive Index (R)
Standard Glass Beads Mie		STD-GLASSBEADS[STD-GLASSBEADS(1.510 - 0.000i),
Standard Glass Beads Fraunhofer		Fraunhofer Kernel[Fraunhofer Kernel(0.000 - 0.000i



Graph Type	D(v,0.1)	D(v,0.5)	D(v,0.9)
	8.98783(µm)	13.47741(µm)	18.8536
	2.58072(µm)	3.62044(µm)	22.3174

Size and size distribution

There are several techniques to determine the size and size distribution of nanoparticles, including:

1. Laser Diffraction Particle Size Analyzer: This technique utilizes the scattering pattern of light beams to measure particle size.

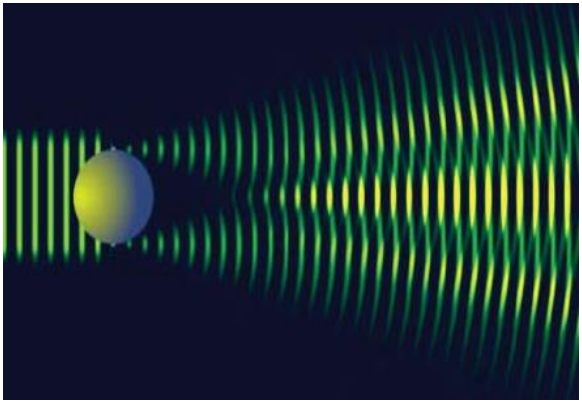
2. Dynamic Light Scattering (DLS): DLS utilizes the Brownian motion of particles in a colloidal suspension to determine their size.

6.
LD – Laser diffraction

The Laser Diffraction Technique - Equipment

In laser diffraction, the particle will scatter light at an angle determined by its size.

A collection of particles will produce a scattering pattern defined by intensity and angle, which can be converted into data on the particle size distribution.



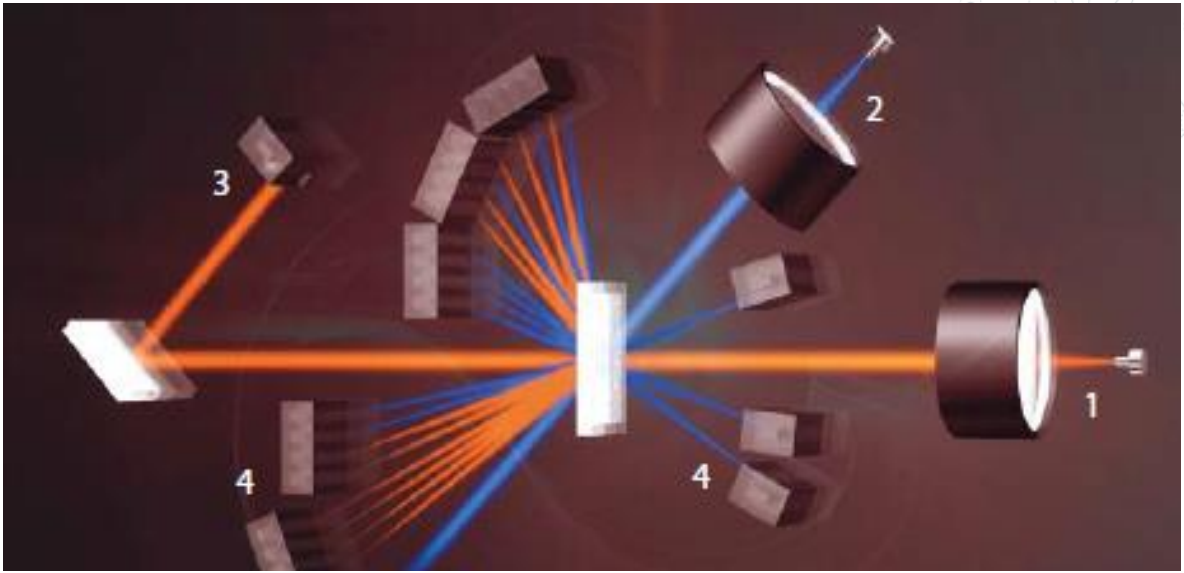
Diffraction pattern of a plane wave being scattered by a spheroid. (Adapted from HORIBA INSTRUMENTS INC., 2016)

The equipment includes the following apparatus:

- The equipment includes at least one high-intensity light source,
- Monochromatic light,
- A sample manipulation system to control the interaction of particles and incident light,
- **A high-quality array of photodiodes to detect scattered light over a wide range of angles.**

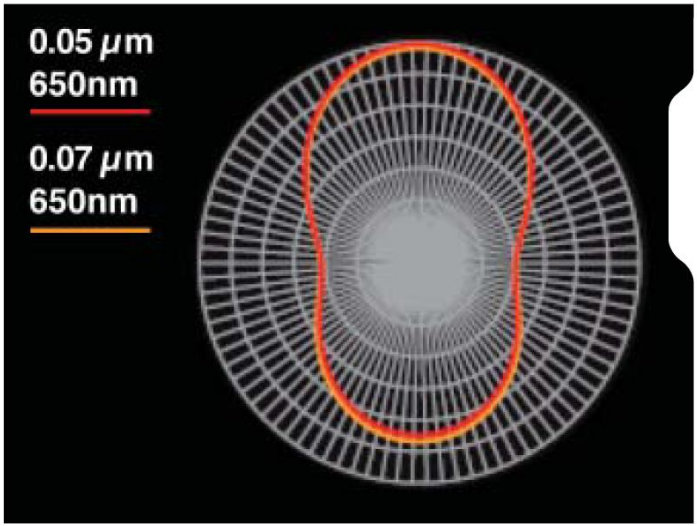
This array is the main apparatus for the laser diffraction instrument as it records the intensity and angle of the scattered light.

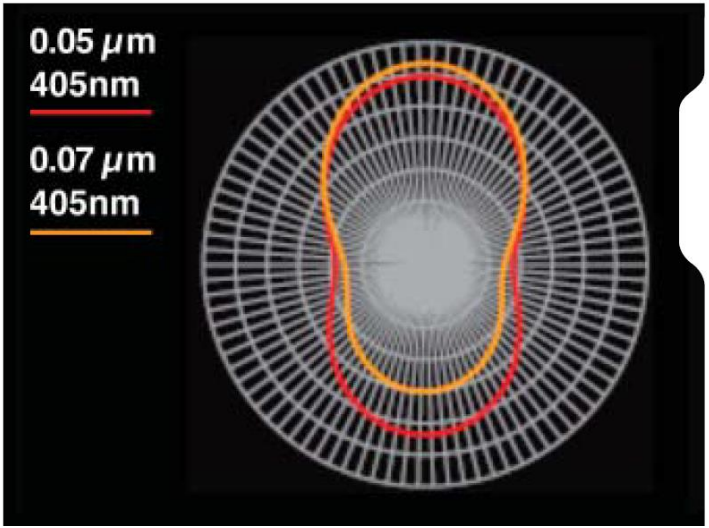
- The gathered information is then input into an algorithm, which consists of an optical model with appropriate mathematical transformations to provide particle size data.



Simplified optical setup found in the laser diffraction instrument model LA-960:

1. Red Laser for particles > 500nm.
2. Blue LED for particles < 500nm.
3. Small-angle detectors for large particles.
4. Side and rear detectors.

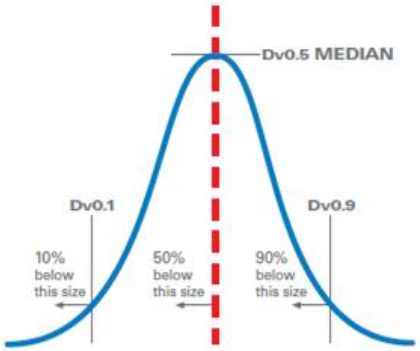

0.05 µm
650nm
0.07 µm
650nm


0.05 µm
405nm
0.07 µm
405nm

Laser diffraction - Interpretation of the calculations

The result of the analysis by the equipment is given by the values of D10, D50, and D90, based on the volume distribution of the particles.

- D50: After the analysis, the median of the found diameters is calculated. This value represents the diameter where half of the particles fall; in other words, half of the particles in the sample have a diameter smaller than the D50 value.
- D90: Following the previous explanation, this value represents the diameter where 90% of the particles in the sample have a diameter smaller than the D90 value.
- D10: Similarly, 10% of the particles have a value smaller than the D10 value.

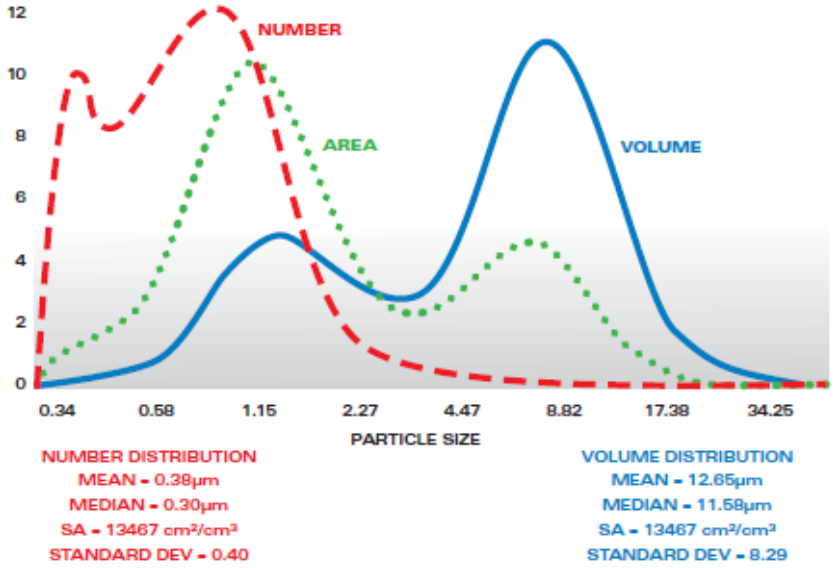


Values on the X-axis for D10, D50, and D90. (Adapted from HORIBA INSTRUMENTS INC., 2016)

Laser diffraction - Interpretation of the calculations

The results obtained from microscopy show values of number distribution, while the results from laser diffraction show values of volume distribution.

There is a possibility of transforming volume distribution values into number distribution, but this conversion is only recommended when comparing the results with microscopy analysis to avoid errors. It is important to note that the two distributions represent different aspects of the particle population, and direct comparison between the two may not always be appropriate without considering the underlying principles and assumptions of each technique. Careful consideration and validation are necessary before making any such conversions or comparisons.



Volume distribution converted to number and area. (Adapted from HORIBA INSTRUMENTS INC., 2016)

7.
DLS – Dynamic light scattering

DLS – Dynamic light scattering

Non-invasive and well-established technique for measuring the size of particles and macromolecules, typically in the submicron and nanometer range.

It is used to measure particles suspended in a liquid.

Examples of applications include proteins, polymers, micelles, carbohydrates, nanoparticles, colloidal dispersions, and emulsions.

Non-spherical particles

The hydrodynamic diameter of a non-spherical particle is the diameter of a sphere that has the same translational diffusion rate as the particle.

For rod-shaped particles, small changes in the length directly affect the size, while changes in the diameter of the rod, which hardly affect the diffusion rate, are difficult to detect.

Conformational changes generally affect the diffusion rate, and DLS is a highly sensitive technique for detecting these changes.

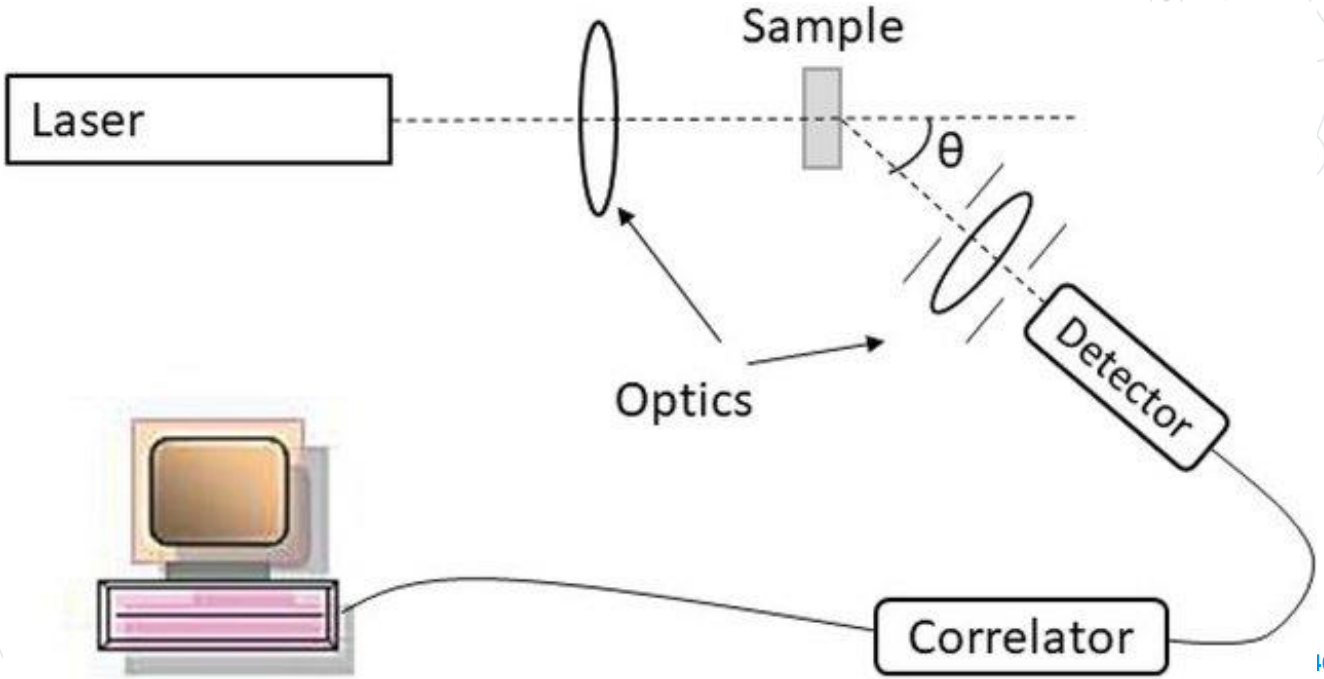
Dynamic Light Scattering (DLS) - How does it work?

Dynamic Light Scattering (DLS) works by determining the rate at which the intensity of scattered light fluctuates when detected using a suitable optical arrangement.

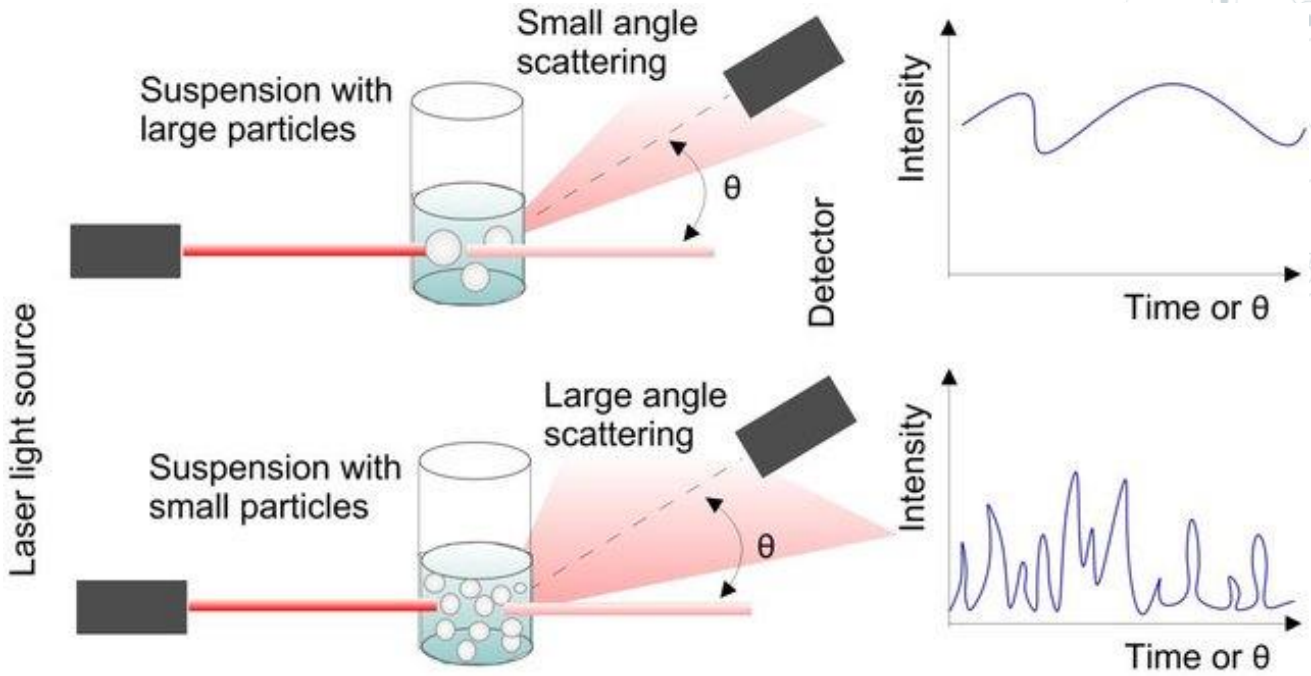
The rate at which these intensity fluctuations occur depends on the velocity and, therefore, the size of the particles.

Smaller particles cause the intensity to fluctuate more rapidly than larger particles.

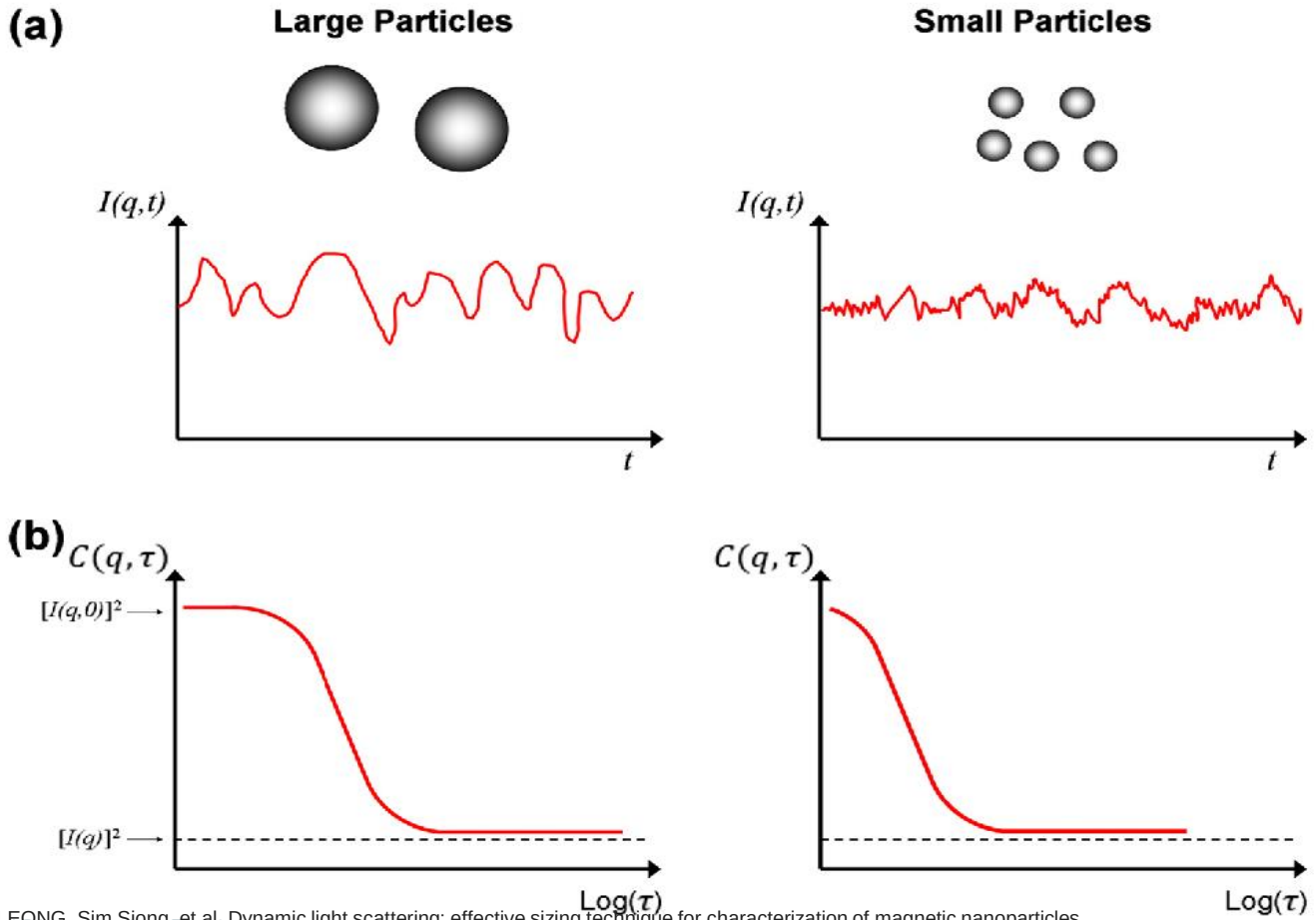
Dynamic Light Scattering (DLS) - How does it work?



DELFINO, Ines; CAVELLA, S.; LEPORE, M. Scattering-based optical techniques for olive oil characterization and quality control. *Journal of Food Measurement and Characterization*, 2019, 13.1: 196-212.

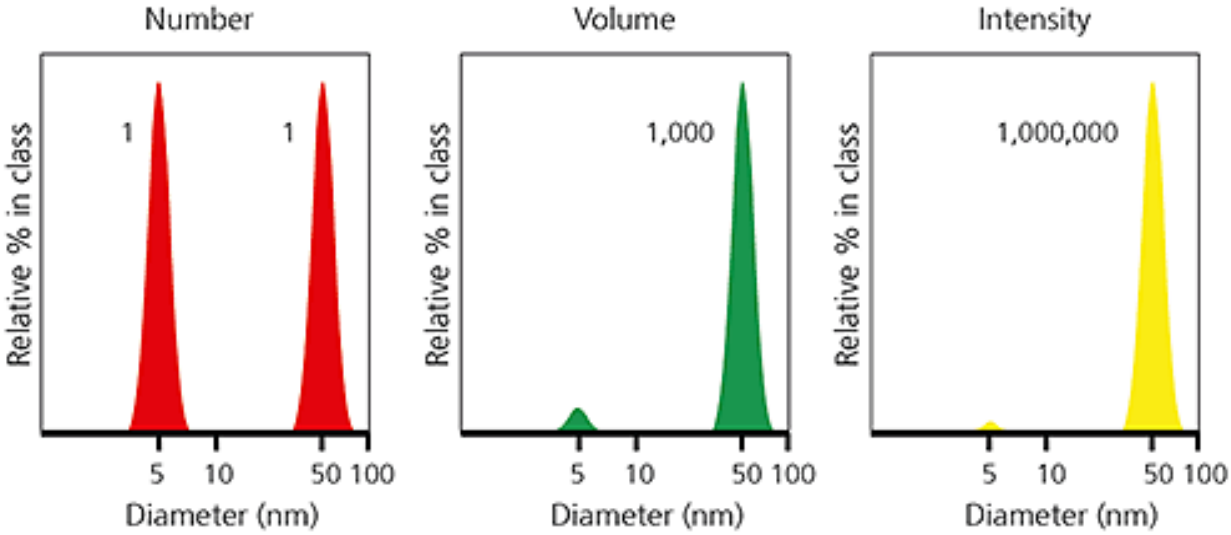


NIKOLOVA, Maria; BAYRYAMOV, Assist Prof Stanislav. A REVIEW OF METHODS AND TECHNIQUES FOR CHARACTERIZATION OF STRUCTURE, MORPHOLOGY AND DISPERSION STABILITY OF MICROCAPSULES. 2019.



LEONG, Sim Siong, et al. Dynamic light scattering: effective sizing technique for characterization of magnetic nanoparticles. In: *Handbook of Materials Characterization*. Springer, Cham, 2018. p. 77-111.

Size information



Zeta potential

ζ

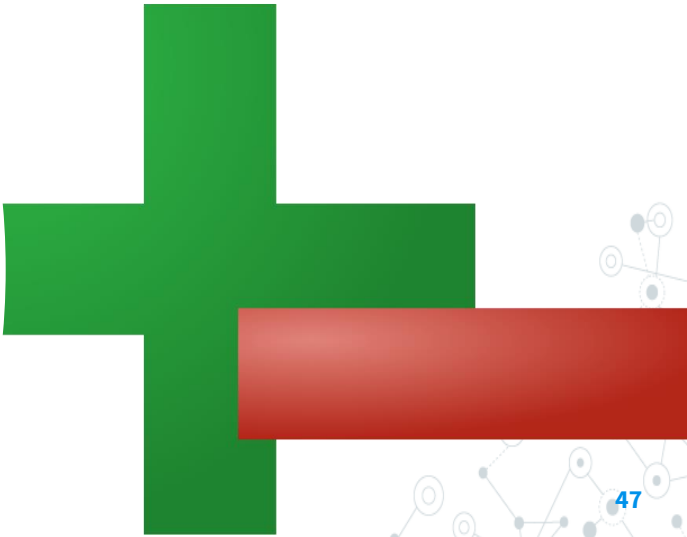
Introduction

Also known as electrokinetic potential, the zeta potential is related to the surface charge of the material.

- Every material has a charge or acquires a charge when suspended in a medium.
- The charge is generated due to the dissociation of functional groups.
- It is pH-dependent.

HATTACHARJEE, Sourav. DLS and zeta potential – What they are and what they are not? **Journal Of Controlled Release**, [S.L.], v. 235, p. 337-351, ago. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.jconrel.2016.06.017. KASZUBA, Michael; CORBETT, Jason; WATSON, Fraser McNeil; JONES, Andrew. High-concentration zeta potential measurements using light-scattering techniques. **Philosophical Transactions Of The Royal Society A**: Mathematical, Physical and Engineering Sciences, [S.L.], v. 368, n. 1927, p. 4439-4451, 28 set. 2010. The Royal Society. http://dx.doi.org/10.1098/rsta.2010.0175. Keck, C.M. and Muller, R.H. (2006) Drug Nanocrystals of Poorly Soluble Drugs Produced by High Pressure Homogeny. European Journal of Pharmaceutics and Biopharmaceutics, 62, 3-16.

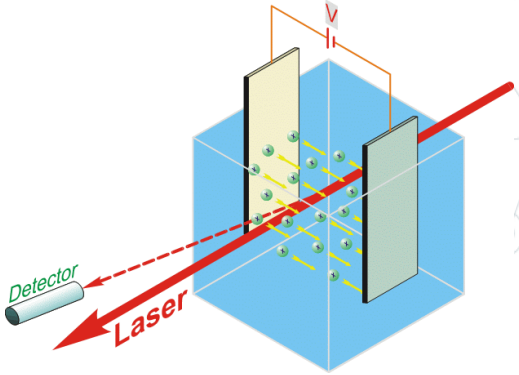
Are the particles in this formulation positive or negative?



Does the product have sufficient electrostatic repulsion to maintain its stability?

Context

Charge Repulsion Stability



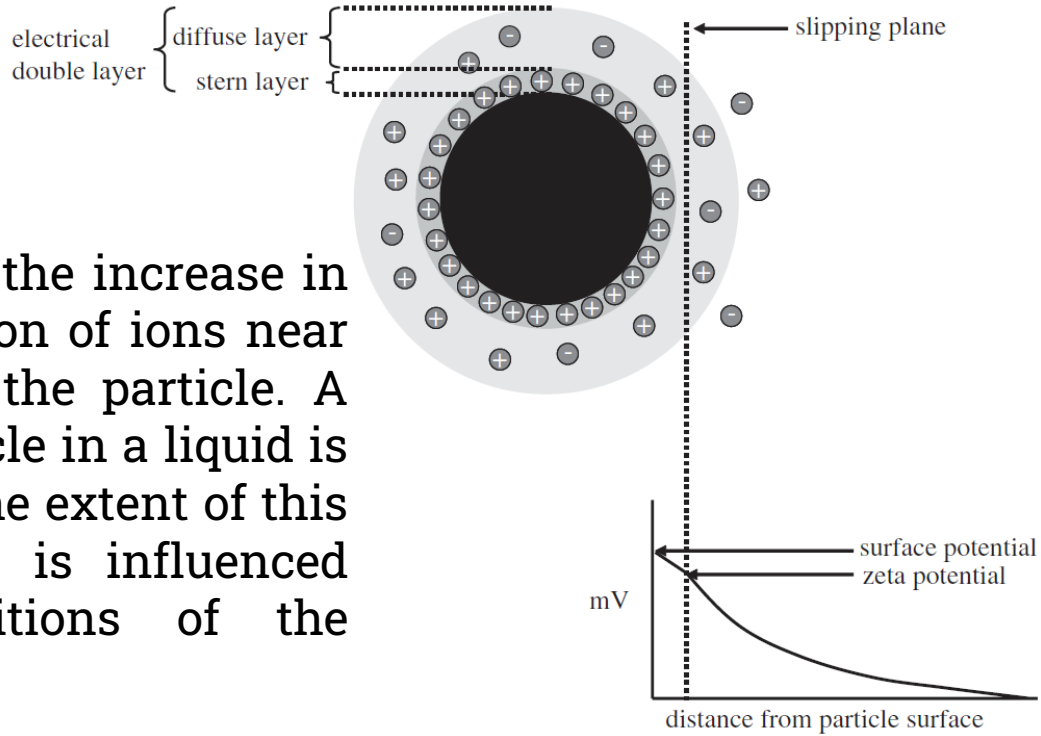
- The zeta potential is measured through the electrophoretic mobility of particles in an electric field.

$$\mu = \frac{v}{E}$$

HATTACHARJEE, Sourav. DLS and zeta potential – What they are and what they are not? **Journal Of Controlled Release**, [S.L.], v. 235, p. 337-351, ago. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.jconrel.2016.06.017. KASZUBA, Michael; CORBETT, Jason; WATSON, Fraser McNeil; JONES, Andrew. High-concentration zeta potential measurements using light-scattering techniques. **Philosophical Transactions Of The Royal Society A**: Mathematical, Physical and Engineering Sciences, [S.L.], v. 368, n. 1927, p. 4439-4451, 28 set. 2010. The Royal Society. http://dx.doi.org/10.1098/rsta.2010.0175. Keck, C.M. and Muller, R.H. (2006) Drug Nanocrystals of Poorly Soluble Drugs Produced by High Pressure Homogeny. European Journal of Pharmaceutics and Biopharmaceutics, 62, 3-16.

The zeta potential is the main parameter controlling electrostatic interactions in particulate dispersions and is crucial for understanding the stability of colloidal dispersions.

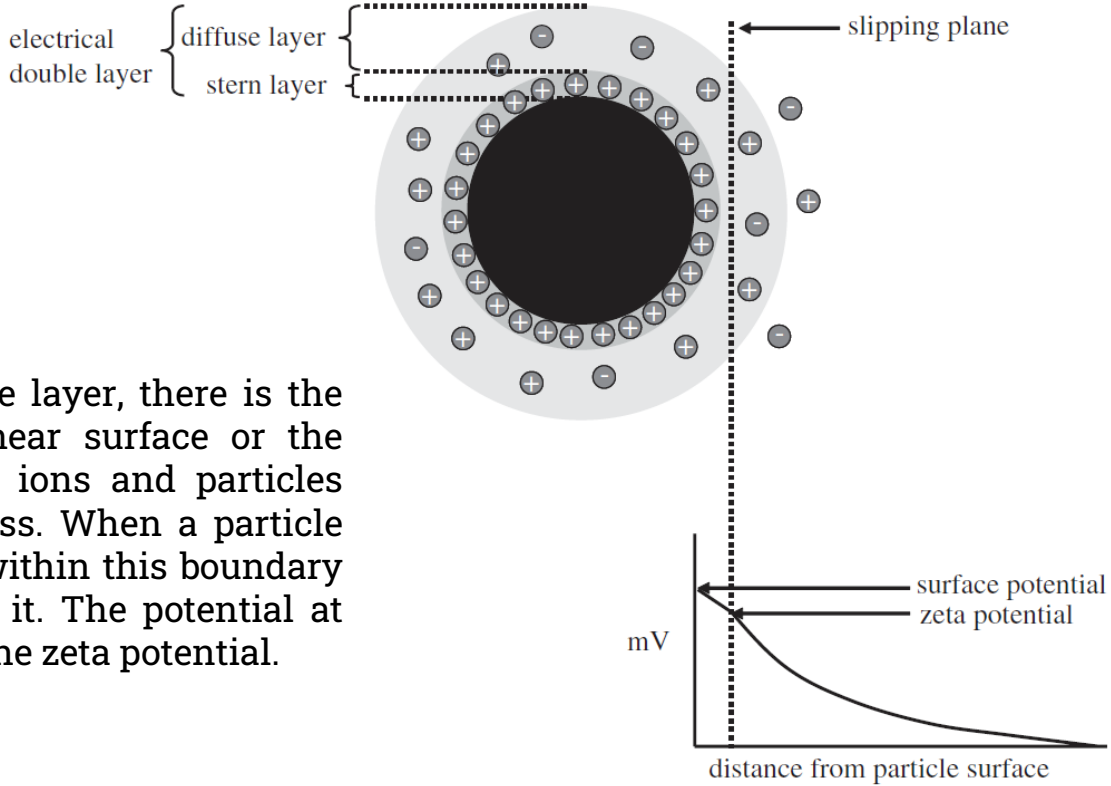
Electrical double layer



It is formed by the increase in the concentration of ions near the surface of the particle. A dispersed particle in a liquid is solvated, and the extent of this solvation layer is influenced by the conditions of the medium

HATTACHARJEE, Sourav. DLS and zeta potential – What they are and what they are not? **Journal Of Controlled Release**, [S.L.], v. 235, p. 337-351, ago. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.jconrel.2016.06.017.
KASZUBA, Michael; CORBETT, Jason; WATSON, Fraser McNeil; JONES, Andrew. High-concentration zeta potential measurements using light-scattering techniques. **Philosophical Transactions Of The Royal Society A**: Mathematical, Physical and Engineering Sciences, [S.L.], v. 368, n. 1927, p. 4439-4451, 28 set. 2010. The Royal Society. http://dx.doi.org/10.1098/rsta.2010.0175. Keck, C.M. and Muller, R.H. (2006) Drug Nanocrystals of Poorly Soluble Drugs Produced by High Pressure Homogeny. European Journal of Pharmaceutics and Biopharmaceutics, 62, 3-16.

Electrical double layer



Within the diffuse layer, there is the hydrodynamic shear surface or the slip plane where ions and particles form a stable mass. When a particle moves, the ions within this boundary move along with it. The potential at this boundary is the zeta potential.

HATTACHARJEE, Sourav. DLS and zeta potential – What they are and what they are not? **Journal Of Controlled Release**, [S.L.], v. 235, p. 337-351, ago. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.jconrel.2016.06.017.
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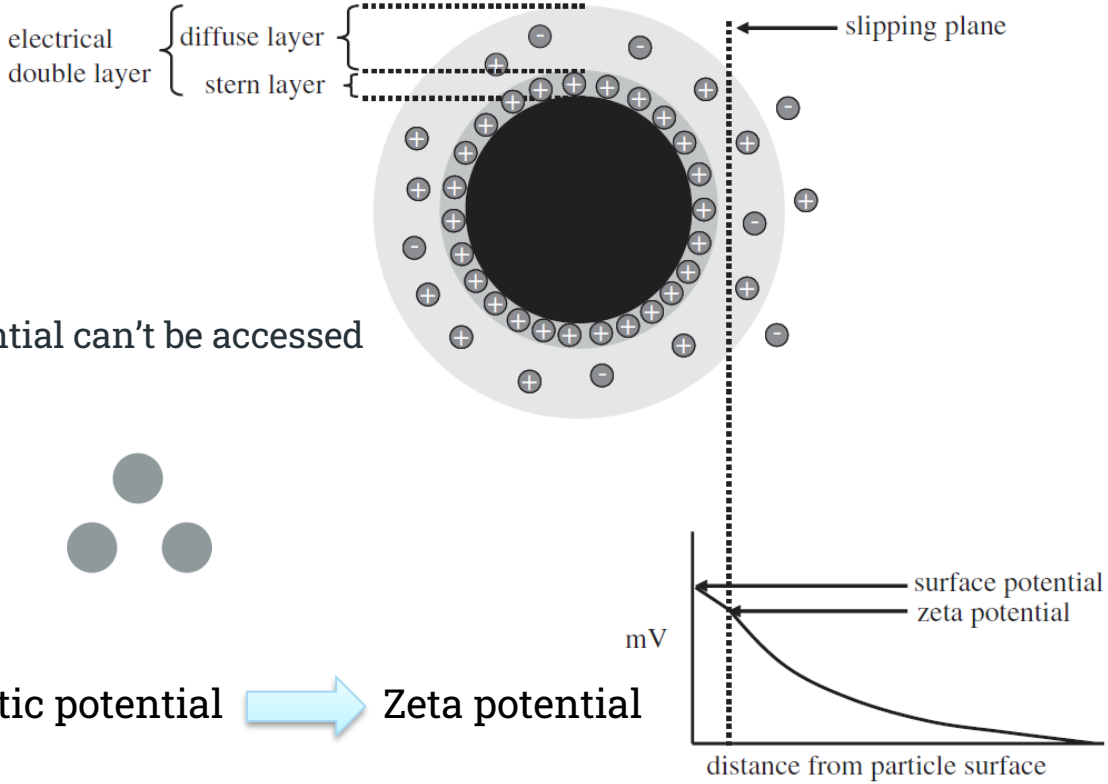
Electrical double layer



- Nernst potential can't be accessed

Electrophoretic potential ⇒ Zeta potential

HATTACHARJEE, Sourav. DLS and zeta potential – What they are and what they are not? **Journal Of Controlled Release**, [S.L.], v. 235, p. 337-351, ago. 2016. Elsevier BV. http://dx.doi.org/10.1016/j.jconrel.2016.06.017.
KASZUBA, Michael; CORBETT, Jason; WATSON, Fraser McNeil; JONES, Andrew. High-concentration zeta potential measurements using light-scattering techniques. **Philosophical Transactions Of The Royal Society A**: Mathematical, Physical and Engineering Sciences, [S.L.], v. 368, n. 1927, p. 4439-4451, 28 set. 2010. The Royal Society. http://dx.doi.org/10.1098/rsta.2010.0175. Keck, C.M. and Muller, R.H. (2006) Drug Nanocrystals of Poorly Soluble Drugs Produced by High Pressure Homogeny. European Journal of Pharmaceutics and Biopharmaceutics, 62, 3-16.

Rule of thumb

ZP > |60| mV:
Very stable formulation

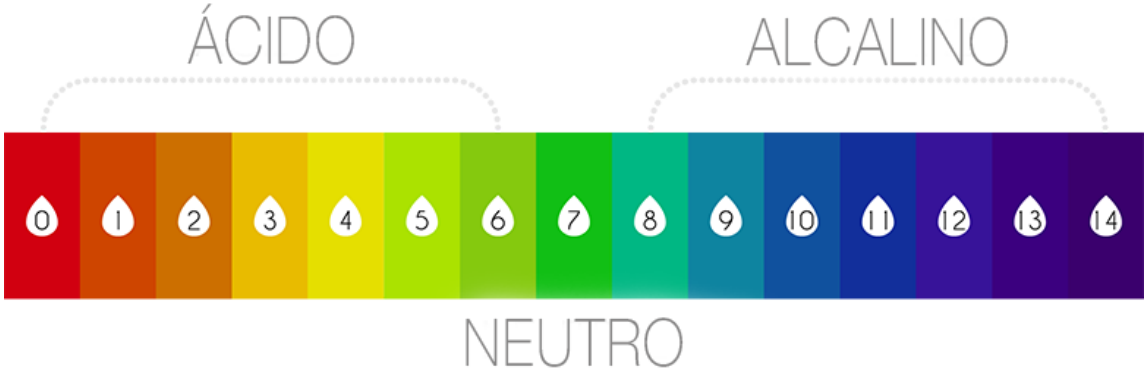
ZP > |30| mV:
Stable formulation.

ZP < |20| mV:
Formulation with partial stability.

ZP < |5| mV:
Unstable formulation susceptible to aggregation.

Factors that influence the zeta potential

pH: The zeta potential without defining the solution conditions is a meaningless number.



Factors that influence the zeta potential

Conductivity:

The thickness of the electrical double layer depends on the concentration of ions in the solution. Higher ionic strength leads to a more compressed double layer.

This double layer can become thicker or thinner depending on the ion concentration in the solution. The higher the ion concentration, the more compressed the double layer, making the ions less accessible for evaluation.

Concentration of formula components:

Knowledge about the components and their concentrations leads to a more stable formulation.

The charge of the components will influence the zeta potential, increasing or decreasing its value. So, it is essential to know the components you are using in the formula development to achieve a more stable formulation.

Measurement

Micro-electrophoresis.

Charged particles are attracted to the electrode with the opposite charge, and the equipment provides information about the value and charge of the formulation. The speed at which the particle is attracted to the electrode is measured and expressed as electrophoretic mobility.

Dynamic light scattering is the main method used.

Henry equation

μ_e = Electrophoretic mobility
ε_r = Dielectric constant
ε_0 = Permittivity of vacuum
ζ = Zeta potential
$f(\kappa\alpha)$ = Henry's function
η = Viscosity

$$\mu_e = \frac{2\varepsilon_r \varepsilon_0 \zeta f(\kappa\alpha)}{3\eta}$$

Henry's equation converts electrophoretic mobility into zeta potential.

The Helmholtz-Smoluchowski equation.

It is applied when the thickness of the EDL is much smaller compared to the particle's radius (typically for larger particles - > 1 μm - in aqueous solutions with a high concentration of salt).

$$\mu_\varepsilon = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta}$$

The Helmholz-Smoluchowski equation applies to most pharmaceutical preparations, and it is of great importance for the development of nanoparticles.

Hückel equation

It is used when the thickness of the EDL is much larger than the particles (particles below 100 nm) dispersed in a low concentration of salt.

$$\mu_\varepsilon = \frac{2\varepsilon_r \varepsilon_0 \zeta}{3\eta}$$

It is usually not relevant for pharmaceutical preparations since it is not applicable to aqueous dispersions.

Thank you!