### Principles, Methods, and Application of Particle Size Analysis

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# Outline

- Dynamic Light Scattering
- Zeta Potential
- Laser Diffraction Analysis





### What is application of particle technology?

Biological, Pharmaceutical, Chemical, Food industrial, Manufactural, etc.



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### Methods for Characterization of Particle Size and Distribution

- ✓ TEM
- ✓ SEM
- ✓ Optical microscopy
- ✓ Sedimentation
- ✓ Light scattering
  - Dynamic light scattering (DLS, measuring fluctuation change of the intensity of the scattered light)
  - Static light scattering (measuring the total intensity of the scattered light)

The size results are depending on the used techniques

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# Laser Light Scattering



Flow cell or closed cell (cuvette)

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## Malvern Zetasizer Ultra



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# Dynamic Light Scattering (DLS)

- Particles suspended in a liquid that are too small to settle will be colliding with the molecules of the liquid and moving about randomly. This is Brownian motion.
- ✓ The fluctuations in scattered light caused by Brownian motion can yield size information.
- Measuring the time dependent fluctuations in the scattering intensity,



Size	<b>Brownian motion</b>	Scattering fluctuation
Small	Fast	Fierce
Large	Slow	Moderate

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#### **Analytical Assumptions and Constraints**

- ✓ The particles are moving in Brownian motion only,
  - ✓ i.e. sedimentation is not occurring.
- ✓ The suspension is stable
- $\checkmark$  The diameter determined is the hydrodynamic diameter (Dh)
- ✓ The viscosity of the suspension is not significantly different from the viscosity of the pure base liquid.



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## **STOKES-EINSTEIN EQUATION**



Brownian motion speed  $\rightarrow$  Particle size

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

- d (H) = hydrodynamic diameter
- D = diffusion coefficient

(Velocity of Brownian motion)

- k = Boltzmann's constant
- T = absolute temperature
- $\eta$  = viscosity of the suspending liquid

### Hydrodynamic diameter



- The diameter measured by DLS correlates to the effective particle movement within a liquid
- Particle diameter +
   electrical double layer
- Affected by surface bound species which slows diffusion

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# **Data Conversion**



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### Size distribution by number, volume, and intensity



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# **Detection Angle**



173° detection optics - Backscatter detection is the most common used for DLS

The application of the Backscatter detection is by a patented technology called NIBS (Non-Invasive Back-Scatter).

Why measure backscatter? There are at least a couple of advantages to doing this:

- The incident beam does not have to travel through the entire sample. This reduces an effect known as multiple scattering, where the scattered light from one particle is itself scattered by other particles. As the light passes through a shorter path length of the sample, then higher concentrations of sample can be measured.
- Contaminants such as dust particles within the dispersant are typically large compared to the sample size.
   Large particles mainly scatter in the forward direction. Therefore, by measuring the backscatter, the effect of dust is greatly reduced.

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#### Sample concentration:

- ✓ Result obtained from DLS should be concentration independent
- Each type of sample material has its own ideal range of concentration where measurement should be made.
- If the concentration is too low, there may not be enough light scattered to make a measurement
- ✓ If the concentration is too high, the result may not be independent of sample concentration
- During method development, determining the correct sample concentration can involve several size measures at different concentration

#### **Lower detection limit**

The lower detection limit of the instrument for concentration and size depends on the intensity of excess scattering produced by the particles

- Size: Intensity of light scattered increases exponentially with size of particles/macromolecules
- ✓ Concentration: Scattering increases with number of particles/macromolecules in solution
- Relative RI: The greater the difference between the materials' refractive index and that of the dispersant, the more the particles/macromolecules scatter

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# **Size Cell Selection**

Part number and Description	ZEN0040 Disposable plastic micro cuvette	DTS0012 12mm o.d. square polystyrene cuvettes	ZSU1002 Low Volume Disposable Sizing Cell Kit	PCS1115 square <b>glass</b> cell with square aperture and cap
Minimum volume	40ul	1 mL	3uL	1 mL
Sample requirement:	BEZANDO		ZSU1002	

- ✓ The sample should consist of well-dispersed particles in a liquid medium
- ✓ The dispersant should meet the following requirements:
- Be sufficiently transparent(non-absorbing) and non-fluorescent at the laser wavelength
- Be free of particulate contamination
- Not dissolve, swell, or coagulate the particulate material
- Have a known refractive index that is sufficiently different from that of the particulate material
- Have a known value of viscosity within ±2% over the operational range of temperature to be used
- Low background scattering

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#### Two different analysis are performed:

Cumulants analysis

- ✓ Mean size (z-average))
- ✓ Polydispersity index

Distribution analysis

commence parameters

✓ Distribution of sizes

#### Adding parameters to the tables



Choose which parameters to display				
Available parameters <b>Q</b> Search			Selected	
Measurement Start Date Measurement Start Time Instrument Serial Number Temperature (°C) Scattering Collection Angle (°) Cuvette Position (mm) Attenuator Cell Name Total Number Concentration (particles/ml) pH Z-Average (nm) Polydispersity Index (Pl) Intercept Peak 1 Mean by Intensity ordered by area (nm) Peak 2 Mean by Intensity ordered by area (nm) Peak 3 Mean by Intensity ordered by area (m) Peak 1 Area by Intensity ordered by area (m) Peak 2 Area by Intensity ordered by area (%) Peak 3 Area by Intensity ordered by area (%) Peak 1 Width by Intensity ordered by area (m)	<ul> <li>Number Peak Summary Data ordered by size</li> <li>Volume Distribution Summary Data Dv (10) Dv (50) Dv (90) Span</li> </ul>		Sample Name Quality Indicator Measurement Type Z-Average (nm) Temperature (°C) Attenuator Measurement Start Time Zeta Potential (mV) Scattering Collection Angle (°) Cuvette Position (mm) Cell Name Total Number Concentration (particles/ml) Mean Count Rate (kcps) Conductivity (mS/cm)	

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#### Sample Contains Large Particles/Aggregates/Contaminants



- Perform at least 3 repeat measurements on the same sample the count rates should be all within a few percent of one another.
- If not, there might be large particles/aggregates/contaminants
- They may not be suitable for measurement by DLS and need to be removed from the sample before re-measurement
- Their presence can caused by/result in:
  - Aggregation
  - Sedimentation
  - Number fluctuations

#### **Results Repeatability**

**Recognising Aggregation and Sedimentation** 



- Increasing Count Rates and Z Average from successive measurements indicate particle aggregation
- **Decreasing** Count Rates and Z Average from successive measurements indicate:
  - Particle dissolution
  - or Sedimentation...

-	j'u column neuact n		
	Sample Name	Mean Count Rate In	Z Average Mean
1	Sample I	136.8	300.5
	Sample II	188.7	309.5
	Sample III	229.7	323.8

Sample Name	Mean Count Rate In	Z Average Mean
Example 1	205.2	166.3
Example 2	184	155.3
Example 3	169.9 0	152.6

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#### **Recognizing Sedimentation**



- If the mean count rate decreases between repeat measurements, the sample will be changing each time it is measured
- · Correlation functions from repeat measurements should perfectly overlap with each other
- If sedimentation is present (or the sample is changing with time), then the correlation functions will not overlap with each other





#### Recognising Number Fluctuations: Useful Parameters

#### Percent In Range

- · Overall indicator of data quality
- Scaled from 0 to 100%
- The higher the value the better ideally > 90%

#### • Intercept

- Signal to noise ratio obtained from the measurement
- 1 = perfect correlation
- Values > 1 caused by number fluctuations (poor definition of the baseline of the correlation function)



#### Parameter Table $\,\, \smallsetminus \,\,$

	Туре	Z Average Mean	Intercept	Percent In Range
2	6			
1	Size	331.1	0.3583	97.05
2	Size	364.9	0.9837	94.62
3	Size	166.3	0.9144	92.55
4	Size	158.4	0.912	88.98
5	Size	155.3	0.9064	93.78
6	Size	154.4	0.9328	93.21
7	Size	154.6	0.9296	94
8	Size	156.3	0.9002	93.04
9	Size	585.4	1.359	83.22
10	Size	181.6	0.6974	92.3

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#### **Data Quality Guidance**

#### **Neural Network Based System for Size Measurements**



• Uses artificial neural network (machine learning) to assess data quality:



Records View Icon	Interpretation
	No data quality issues found.
0	The data is useable, but advice should be followed when using this data. e.g. Multiple populations – the distribution analysis is a meaningful description of the results and the Z-average cannot be easily interpreted
	The data is of poor quality and the remedial advice should be followed before using data from this sample.

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### **Data Quality Guidance**

Providing Advice for improved quality of the measurement

- A fluorescently tagged lipid nanoparticle sample was measured using all the set up at default.
- Data quality advice indicated the poor quality attributed due to flare or fluorescence



- The fluorescence filter excluded the fluorescence noise
- Improved the quality of the data
- Correlation coefficient increased

#### Malvern Panalytical a spectris company

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### Summary of Dynamic Light Scattering (DLS)

Advantage	Limitation
Wide particle size range (0.3 nm - 10 μm)	Stable suspension is required
Small volume of sample (3 $\mu$ l)	
Independent result from refractive index	
Minimum sample concentration depending on sample and size (0.1 mg/mL)	
Recovery of sample	
Reliable result	

# **Zeta Potential**

#### Colloidal/Dispersion Stability

- Colloidal stability determined by the sum of attractive forces (van der Waal) and repulsive force (electrostatic)
- Zeta potential is a measure of the electrostatic or charge repulsion present in the sample
- ✓ Stability can be lost through various mechanisms



https://doi.org/10.1016/j.ijpx.2018.100002

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# Maintaining Colloidal Stability

Two mechanisms can be used to maintain colloidal stability

#### **Steric effect**

#### **Electrostatic effect**

✓ Simple✓ Irreversible

Easy to measure the controlling parameter (Zeta potential) Reversible



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**Zeta Potential**: a measure of the effective electric charge on the nanoparticle's surface. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system.



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High zeta potential = stable dispersion



Low zeta potential = unstable colloid



Zeta Potential (mV)	Stability
0 to ±10	Highly unstable - rapid coagulation/flocculation
±10 to 20	Limited stability
± 20 to 30	Moderately stable
> ± 30	Highly stable

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### Measurement of Zeta Potential





Laser Doppler Velocimetry (LDV)

- Electrophoresis is movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field.
- ✓ It is the electrophoretic mobility that we measure directly with the conversion to zeta potential being inferred from theoretical considerations.

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Zeta potential is just as much a function of the medium as it is the nature of the surface of the particle. It will be dependent on:

- ✓ pH value of medium
- ✓ Ionic strength
- Additive concentration (if the sample is too turbid, dilute is needed.)

### **The Henry Equation**

$$U_e = \frac{2\varepsilon z f(\kappa a)}{3\eta}$$

where z is the zeta potential, Ue is the electrophoretic mobility,  $\epsilon$  is the dielectric constant,  $\eta$  is the viscosity of the dispersant, f(ka) is the Henry function.

Sample limitations:

To measure zeta potential, we need an excess of scattered light. This depends on:

- ✓ Concentration enough particles to scatter the light
- ✓ Size larger particles scatter more light (> 3.8 nm)
- ✓ Refractive index contrast the larger the difference between the refractive index of the dispersant and the particles, the more excess scattering
- ✓ Zeta potential is measured using a forward angle of detection; therefore, the laser beam must pass through the entire sample.
- Too high a concentration and the beam becomes attenuated by the sample and the amount of signal detected is reduced.
- ✓ Zetasizer Advance uses an attenuator to adjust the transmission of the light

Very conductive samples could be challenging (such as protein) due to:

- ✓ Joule heating
- ✓ Electrode polarization
- ✓ Sample degradation
- ✓ Electrode degradation
- ✓ Maximum conductivity that can be measured is 260 mS/cm

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### Cell selection:

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- ✓ DTS 1070 disposable folded capillary plastic cell for use in aqueous systems
- ✓ ZEN1002 dip cell used for both aqueous and non-aqueous





Quartz glass square cuvette

### Summary of Zeta Potential

Size range suitable for measurement	3.8 nm – 100 μm
Mobility range	> +/- 20 μ.cm/V.s
Minimum sample volume	20 µL
Minimum sample concentration	1 mg/mL
Maximum sample concentration	40% w/v
Maximum sample conductivity	260 mS/cm

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### Malvern Mastersize 3000+ Ultra



For big and insoluble wet and dry particles



True nanometer particle	Quasi nanometer particle	Micrometer particle
Size < λ/10 λ = 633 nm	Size > $\lambda/10$	Size > $\lambda$
Not angle- dependent	Angle dependent	Heavily angle dependent
Elastic scattering (Rayleigh scattering)	Inelastic scattering (Mie scattering)	Inelastic scattering (Mie scattering)









**Dynamic light scattering (DLS)** 

Static light scattering (Laser diffraction)

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# **Mie Theory**

**Mie theory** is a model that predicts the relative intensity of scattered light based on particle size, wavelength, polarization, and angle of observation. The theory is valid for smooth, spherical particles that are internally homogeneous and have a known refractive index. The original theory was developed by Gustav Mie and used a homogeneous sphere model, but it has since been applied to other particle systems, such as cylinders, rods, and concentric spheres.

#### Scattering intensity = Function (particle size, collection angle, wavelength, refractive index, etc.)





Gustav Mie (1868-1957)

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## How is the particle size determined?



When the simulation is perfectly matching to the experimental result, the particle size information could be obtained. Accurate refractive index of the particle is critical.

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## Malvern Mastersize 3000+ Ultra Optics



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# **Radius of Gyration**

Radius of Gyration (Rg): the distance from the center of mass of a body at which the whole mass could be concentrated without changing its moment of rotational inertia about an axis through the center of mass

- ✓ Both Rg and Rh can be used to gain insight into the protein structure.
- Rg is slightly more dependent on the structure of the molecule of interest than the value of Rh.
- ✓ The ratio of Rg and Rh (Rg/Rh) that really provides shape information about a protein molecule. The characteristic Rg/Rh value for a globular protein is ~0.775, which means that Rg is smaller than Rh.
- ✓ However, when molecules deviate from globular to non-spherical or elongated structures then Rg/Rh tend to values upwards of 0.775, as Rg becomes larger than Rh.



#### $R_g$ and $R_h$ are always in the same order of magnitude.

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dry powder disperser



Sample addition and dispersion



Wet sample disperser

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Advantage	Limitations
Wide particle size range (0.01 - 3500 μm)	Big sample amount( > 5 mg or 100 mL)
Measurement is rapid (10 seconds)	Accurate refractive index of the particle material is required.
Friendly software (ZS Xplorer)	Time consuming for cleanup
Suspensions, emulsions, dry powders	High concentration causes multiple scattering

# Thanks for your attention

# Questions?

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