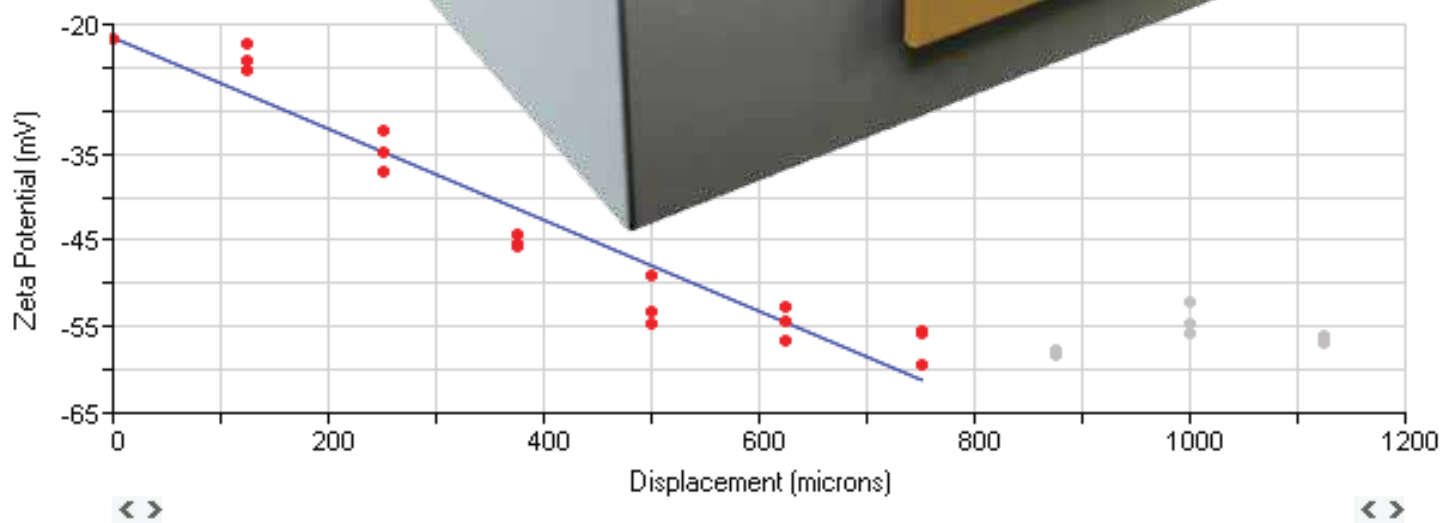
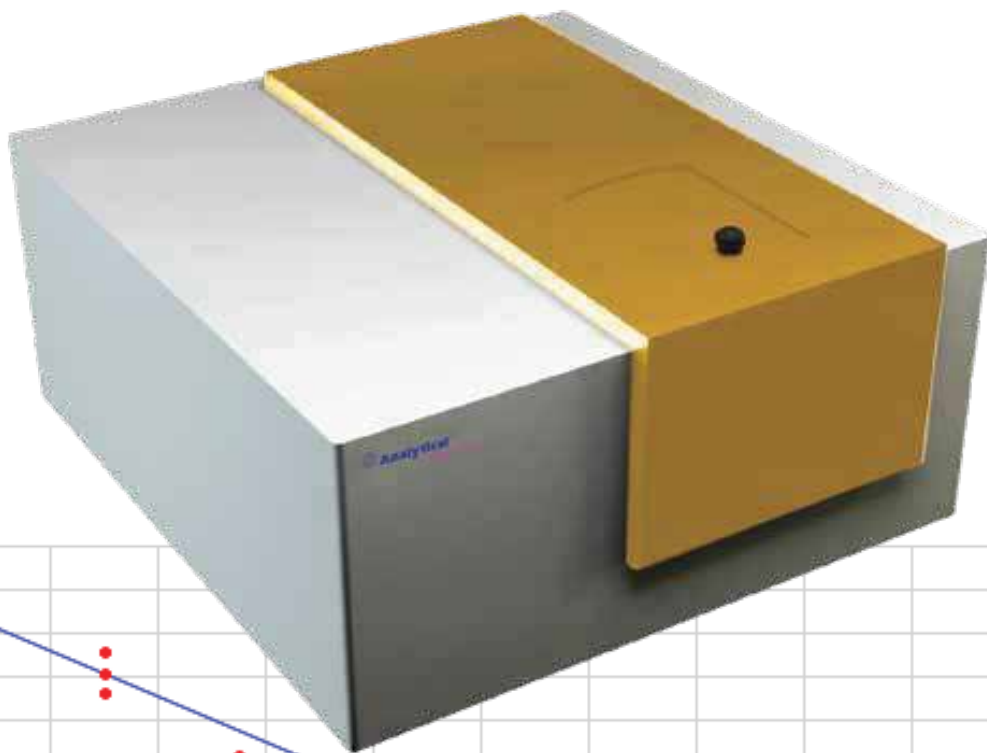


NanoZeta



EPC / PRODUCTS / APPLICATION / SOFTWARE / ACCESSORIES / CONSUMABLES / SERVICES

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►► Why Light Scattering?

The scattering intensity is a function of the molecular weight and concentration. Non-invasive technique, giving information on the size, mass, and charge of a protein sample. Light scattering is extremely sensitive to the presence of small amounts of aggregates. The velocity of a particle under an applied electric field is proportional to the charge.

►► Zeta Potential Principle

- It is a scientific term in colloidal dispersion, in this chemistry it is usually denoted using the greek letter Zeta, hence Zeta Potential. Units(usual) = volts or milivolts.
- It is a electric potential in interfacial double level at the location of sleeping plain relative to point in the bulk fluid away from the interface. Thus, Zeta Potential is the difference between dispersion medium and the stationary layer of fluid attached to the dispersed particle.

►► Introduction to Protein Charge

- The charge acquired by molecule in given medium is its Zeta Potential and getting from surface and the concentration & type of ions in the solution. Particles with equal charge will repel each other, those with high charges will resist aggregation for longer time making such sample stable.
- It means, stability can differ by changing pH, concentration, type of ions & additives.
- **Application :**
 - Protein concentration rises while maintaining stability
 - Development time will reduce for stable disperssion & protein solution.
 - Understanding the product stability in increasing products life.

►► Measurement of Zeta Potential

- The particle charge & molecules is determined by measuing velocity when they are moving due to electrophoresis. These Zeta Potential will migrate towards an electrode if a field is applied. The speed they move is proportional to the field strength and their potential. By knowing field strength, measurement of speed of movement will get using laser & then apply theroies to calculate Zeta Potential. The whole procedure of measurement is simply automated process.

►► Surface Zeta Potential

- Surfaces in contact with a liquid containing ions can have zeta potential in the similar way as dispersed particles and molecules.
- This charge on a surface will repel molecules in liquid & of this can be used for various application. For Eg : In development of contact lens.

►► Molecular Weight Measurement

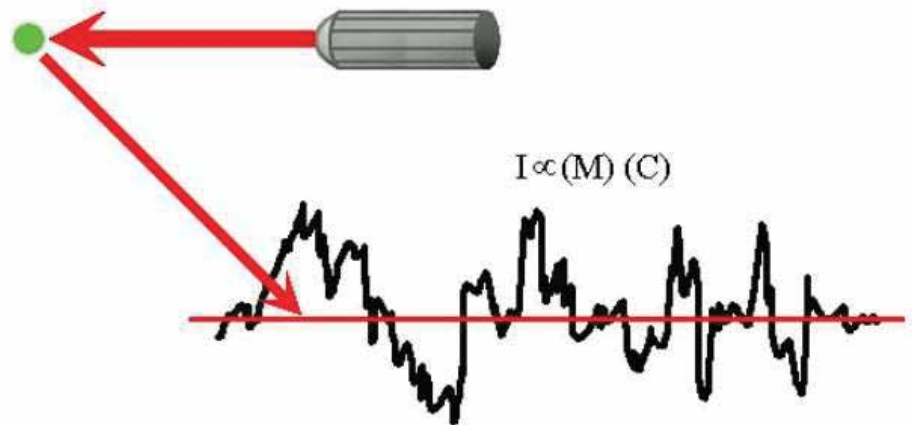
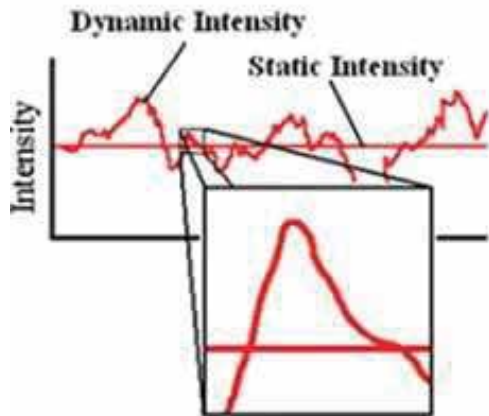
Introduction (SLS):

- This series ensure you to measure molecular weight of macro molecules in solution using Static Light Scattering(SLS). ($I \propto (M_w)(C)$) For this technique product should be stable, fast & sensitive, which has been designed in this instrument.

►► How it measures?

SLS requires the determination of the scattering intensity of number of known concentrations of the macro molecule in solution.

SLS requires the determination of the scattering intensity of number of known concentrations of the macro molecule in solution.



Rayleigh Equation

$$\frac{KC}{R_{\theta}} = \left(\frac{1}{M} + 2A_2C \right) \frac{1}{P(\theta)}$$

K = Optical constant

M = Molecular weight

A_2 = 2nd Virial coefficient

C = Concentration

R_{θ} = Rayleigh ratio

$P(\theta)$ = Shape (or form) factor

$$\boxed{\frac{KC}{R_{\theta}}} = \left(\frac{1}{M} + 2A_2C \right) \boxed{\frac{1}{P_{\theta}}}$$

$$K = \frac{2\pi^2}{\lambda_o^4 N_A} \left(n_o \frac{dn}{dc} \right)^2$$

λ_o = laser wavelength

N_A = Avogadro's number

n_o = Solvent RI

dn/dc = differential RI increment

$$P\theta = 1 + \frac{16\pi^2 n_o^2 R_g^2}{3\lambda_o^2} \sin^2 \left(\frac{\theta}{2} \right)$$

R_g = Radius of gyration

θ = Measurement angle

$$R_{\theta} = \frac{I_A n_o^2}{I_T n_T^2} R_T$$

I_A = Intensity of analyte (sample I – solvent I)

n_o = Solvent RI

I_T = Intensity of standard (toluene)

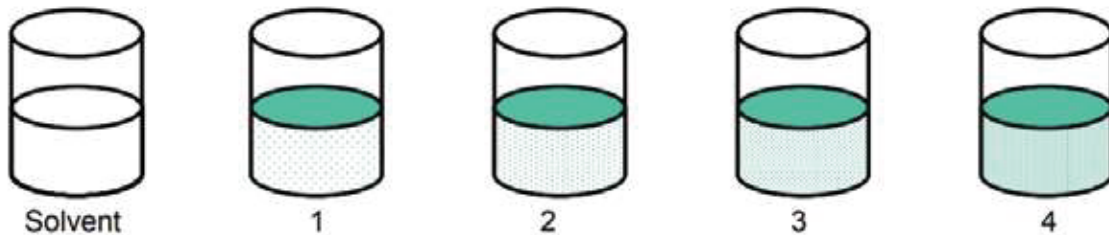
n_T = Standard (toluene) RI

R_T = Rayleigh ratio of standard (toluene)

- For molecules which show no angular dependence in their scattering intensity, accurate molecular weight determinations can be made at a single angle (Rayleigh scatterers, isotropic scattering)
- This is called a Debye plot and allows for the determination of :
 Absolute Molecular Weight
 2nd Virial Coefficient (A_2)

►► Debye plots: What do the measurements involve?

- Preparation of a number of concentrations of the unknown molecule (protein) in a suitable buffer.



Typical concentrations: 1, 2, 3 and 5 mg/mL

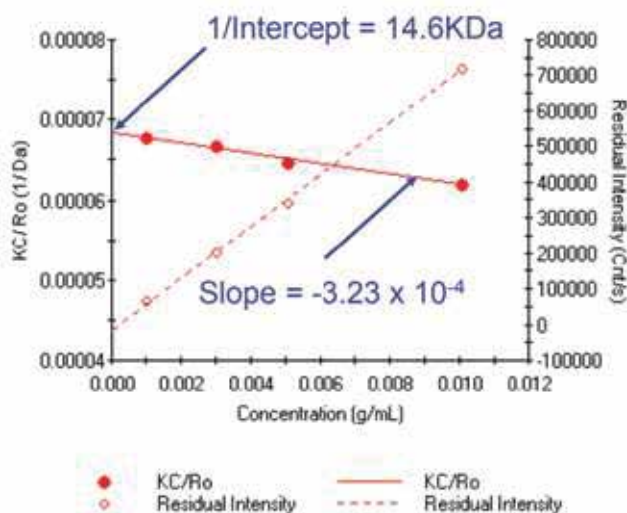
►► Static Light Scattering (SLS)

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C \right) \frac{1}{P_\theta}$$

For Rayleigh scatterers, $P(\theta) = 1$ and the equation is simplified to

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C \right) \quad (y = b + mx)$$

Therefore a plot of KC/R_θ versus C should give a straight line whose intercept at zero concentration will be $1/M$ and whose gradient will be A_2



2nd virial coefficient

A thermodynamic property describing the interaction strength between the molecule and the solvent

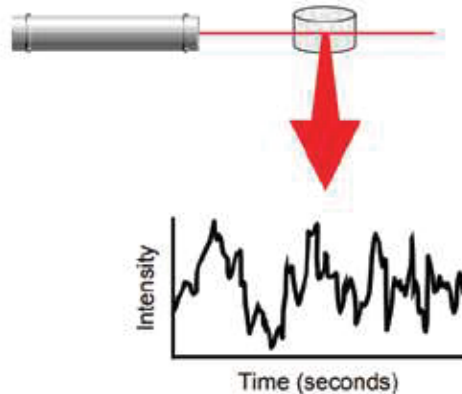
For samples where $A_2 > 0$, the molecules tend to stay in solution (protein molecules prefer contact with buffer)

When $A_2 = 0$, the molecule-solvent interaction strength is equivalent to the molecule-molecule interaction strength – the solvent is described as being a theta solvent (protein doesn't mind buffer)

When $A_2 < 0$, the molecule will tend to fall out of solution or aggregate (protein doesn't like buffer)

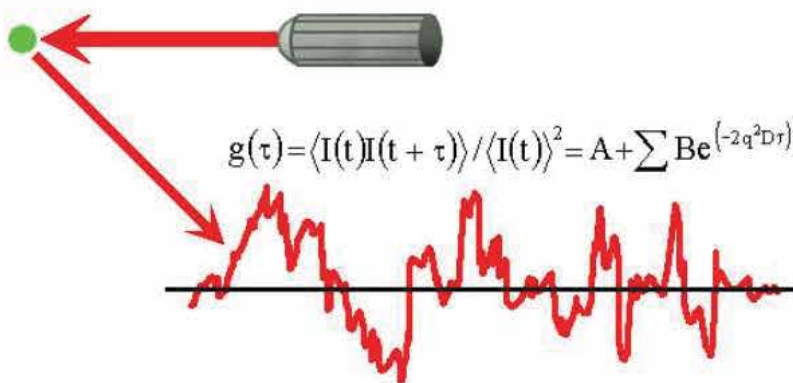
►► Dynamic Light Scattering (DLS)

- Dynamic light scattering is a technique for measuring the size of molecules and nanoparticles.
- DLS measures the time dependent fluctuations in the scattering intensity to determine the translational diffusion coefficient (D_T), and subsequently the hydrodynamic radius (R_H)
- DLS measures the time dependent fluctuations in the scattering intensity to determine the translational diffusion coefficient (D), and subsequently the hydrodynamic size
- The rate of intensity fluctuation is dependent upon the size of the particle/molecule



►► Measurement of molecular size by Dynamic Lights Scattering (DLS)

- Brownian motion : DLS principle is fine particles and molecules are in constant random thermal motion which diffuse at a speed related to their size, smaller particles diffusing faster than larger particles. Brownian motion's is temperature dependent, therefore precision in temperature control is crucial for exact size measurement.
- Measurement of diffusion speed : The speckle pattern produced by illuminating the particles with a laser is observed. So, that intensity at a particular angle will change with time & this is detected using photodiode detector. After this procedure these changes are analysed with a digital co-relator which generate co-relation function. The curve will analysed and provide size and size distribution. To generate quality data, this Zeta Potential is designed to provide optimized component at every stage in the measurements.
- Fluctuations are a result of Brownian motion and can be correlated with the particle diffusion coefficient and size.



Stokes-Einstein

$$R_H = \frac{kT}{6\pi\eta D}$$

q = Scattering vector
 R_H = Radius
 T = Temperature

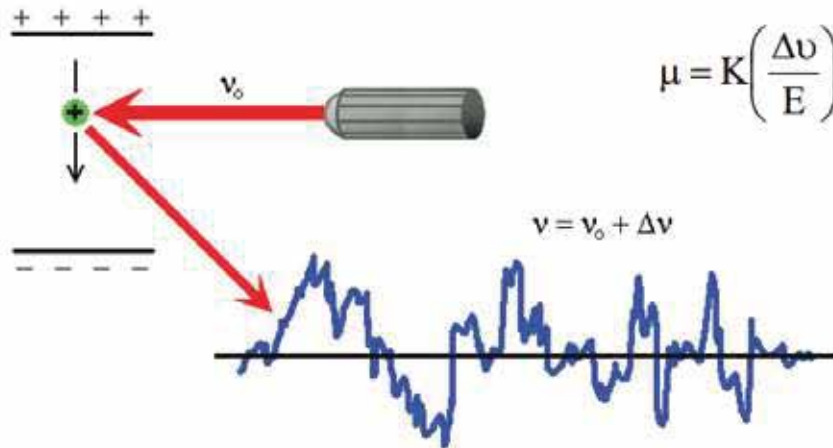
D = Diffusion coefficient
 k = Boltzmann constant
 η = Solvent viscosity

►► Benefits Of Sizing By DLS :

- Non-invasive
- High sensitivity (< 0.1 mg/mL for typical proteins)
- Low volume
- Scattering intensity is proportional to the square of the protein molecular weight, making the technique ideal for identifying the presence of trace amounts of aggregate.

►► Electrophoretic Light Scattering (ELS)

Measured parameter is the frequency shift ($\Delta\nu$) of the light scattered from a moving particle.



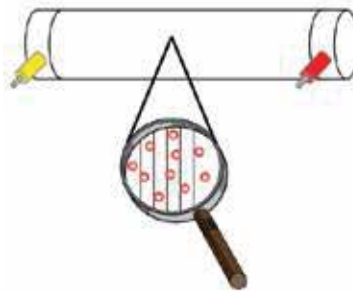
μ is the electrophoretic mobility, E is the electric field strength, and K is a constant

Measuring Electrophoretic Mobility

Classical capillary electrophoresis (light microscope, stopwatch)

The particles move with a characteristic velocity which is dependent on:

- Field strength
- Dielectric constant of medium
- Viscosity of the medium
- Zeta potential



Electrophoresis :

- Electrophoresis is the movement of a charged particle relative to the liquid it is suspended in under the influence of an applied electric field.
- The electrophoretic mobility of a colloidal dispersion can be used to determine the zeta potential
- Zeta potential is the charge a particle acquires in a particular medium.
- Zeta potential measurements can be used to predict dispersion stability.
- Influenced by: pH, salts, concentration, additives,...

Measuring Electrophoretic Mobility

- Laser Doppler electrophoresis (LDE)
 - Phase analysis light scattering (PALS)
 - Mixed mode measurements (M3)
- A laser beam is passed through the sample in the capillary cell undergoing electrophoresis
- Scattered light from moving particles is frequency shifted
- These small frequency shifts are measured
- The frequency shift Δf is equal to:

$$\Delta f = 2v \sin(\theta/2)/\lambda$$

v = the particle velocity
 λ = laser wavelength
 θ = scattering angle

...measure phase instead

▶▶ SIZING

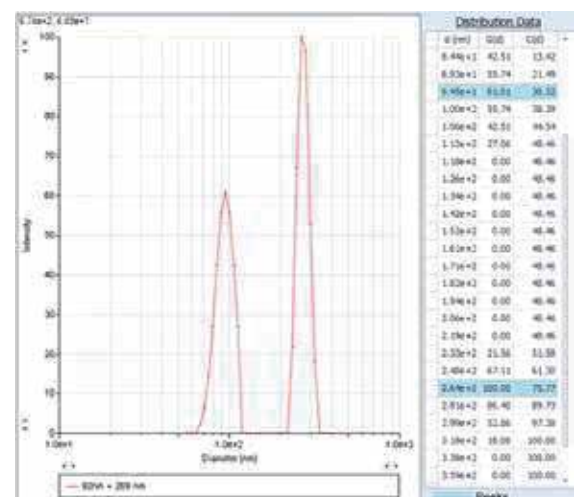
- Rapid and accurate nanoparticle size distributions
- Multimodal & unimodal size distribution software
- ISO 13321 and ISO 22412 compliant results
- Range: > 0.3 nm to 10 μ m
- Three measurement angles: 15°, 90°, & 173°
- High power 35 mW diode laser
- Dynamic light scattering at 173° and 90°
- Temperature control, -5 °C to 120 °C
- Compact bench top unit, USB connection
- Molecular weight determination (relative and absolute through Debye plot)
- Accuracy: $\pm$ 5 % on NIST traceable standards

▶▶ ZETA POTENTIAL

- Zeta potential for the difficult cases
- For proteins, peptides, mAb, RNA, and other biological samples
- For zeta potential in organic solvents
- For oily or viscous media
- For high-salt suspensions
- For samples near the I.E.P.
- 1,000 times more sensitive than other techniques
- Disposable cuvettes, no contamination or alignment
- Built in automatic procedures and parameters.

►► Rapid, Reliable, and Accurate Analysis

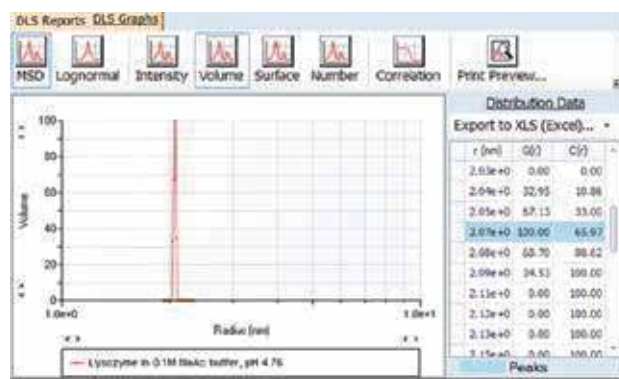
The new NanoZeta Unique particle size and zeta potential analyzer incorporates all you need for fast, routine, submicron measurements of size and zeta potential. Based on the principles of Dynamic Light Scattering (DLS) for particle sizing and distribution, and on Doppler velocimetry (electrophoretic light scattering, ELS) for zeta potential, most measurements only take a minute or two. The instrument also includes Phase Analysis Light Scattering (PALS) measurements for samples with low mobilities (saline, PBS).



►► Three Scattering Angles:

Measurements of traditional colloids are usually made at 90° scattering angle due to the unbiased results measured. For nanoparticles and proteins, peptides, these < 50 nm samples can be measured using the backscattering angle (173°) for best S/N and reproducibility of measurements. Finally the 15° detection angle can be selected for added sensitivity with aggregation measurements. Zeta potential measurements are always performed using the 15° detection angle to minimize diffusion broadening.

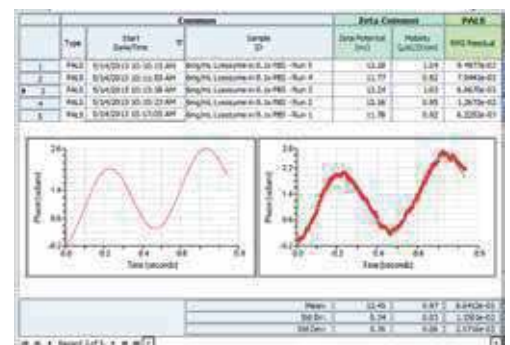
►► Principles of Operation - Sizing



Dilute suspensions, on the order of 0.0001 to 1.0% v/v are prepared, using suitable wetting and/or dispersing agents, if required. A small ultrasonicator is sometimes useful in breaking up loosely-held agglomerates. At 173 sample volume may be reduced to 50 µL with a polystyrene, U-shaped, disposable cuvette and the sample is recoverable. At 90° square polystyrene or glass cells (two or three mL) are used, one as small as 5µL (non-disposable). In addition, disposable, glass round cells with reusable Teflon stoppers are used for aggressive solvent suspensions. In all case, just a few minutes are required for the sample and cell to equilibrate with the actively controlled temperature environment inside the Analytical 3000 Series.

►► Principles of Operation - Zeta Potential

The NanoZeta Unique utilizes phase analysis light scattering to determine the electrophoretic mobility of charged, colloidal suspensions. Unlike its cousin, Laser Doppler Velocimetry (LDV, [sometimes called Laser Doppler Electrophoresis, LDE), the PALS technique does not require the application of large fields which may result in thermal problems or denaturation, because in the measurement of phase shift, the particles need only to move a fraction of their own diameter to yield good results. In salt concentrations up to 2 molar and with electric fields as small as 1 or 2 V/cm enough movement is induced to get excellent results. In addition, the Autotracking feature compensates for thermal drift.



Specifications

Sample Type	Sizing: nano particle and colloidal-sized materials, in any non-absorbing liquid. Zeta potential: proteins, nano particle, polymer and colloidal-sized materials, suspended in any non-absorbing liquid, with relative permittivity (dielectric constant) > 1.5 and viscosity < 30cP.
Size Range	Sizing: > 0.3 nm to 10 µm diameter, depending on refractive index and concentration Zeta potential: 1 nm to 100 µm, sample dependent
Mobility Range	10-20µ.cm to 10-7 m ² /V*s
Zeta potential range	-500 mV to 500 mV, sample dependent
Maximum sample conductivity	Sizing: unlimited Zeta potential: 30-200 mS/cm
Sample Cells	Sizing: 1 to 3 mL disposable plastic, 50 µL disposable, 40 µL quartz flow cell, 5µL quartz minimum Zeta potential: 180 µL, 600 µL, 1250 µL
Concentration Range	Sizing: 0.1 ppm to 50 mg/mL, depending on refractive index and concentration Zeta potential: 40% v/v, sample dependent
Signal Processing	Sizing: Dynamic Light Scattering, DLS Zeta potential: Electrophoretic & true Phase Analysis Light Scattering, ELS & PALS
Correlator	Research grade with more than 510 channels, covering the equivalent of 1010 linearly-spaced channels, 100% efficiency, real-time operation over the entire delay-time range.
Precision	Sizing: ± 1% typical Zeta potential: plus minus 3% typical
Temperature Control	-5 ° C to 120 C, ± 0.1 ° C, active control. No external circulator required.
Condensation Control	Purge facility using dry air, nitrogen preferred
Standard Laser	4-35 mW red diode laser, nominal 600 – 632.8 nm wavelength
Scattering Angle	165° -173°, 90° & 15° / 13°
Data Presentation	Average & width, lognormal fit, and multimodal size distribution for sizing Doppler Frequency Shift, electrophoretic mobility.
Compliance	ISO13321 and ISO22412 compliant results for sizing
Power Requirements	100/115/220/240 VAC, 50/60 Hz, 150 Watts
Dimensions	23.3 x 42.7 x 48.1 cm (HWD)
Weight	15 kg
Detector	High resolution Avalanche Photo Diode
Environmental Characteristics	Temperature 10 ° C to 75 ° C Humidity 0% to 95%, non-condensing
Software	software comes with a user-friendly interface, results previews, and various types of report pages

►► Servicing, Validation, Trainings and Preventive Maintenance :

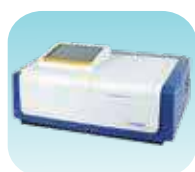
Servicing	: We have team of service engineers who can attend to any make of instrument promptly @the most affordable cost.
Trainings	:We also take up preventive maintenance to reduce downtime of instrument's Trainings.
AMC's/CMC	: We offer user training both in-House and at customer sites on instrument principles, operations, troubleshooting.
Validations	: We have protocols for carrying out periodic Validations as per GLP/ GMP/USFDA norms.
Instruments	: We offer instruments/Renting Services Modules like pumps, detector etc. on Rent.



►► About Analytical Technologies

Analytical Technologies is synonymous for offering technologies for doing analysis and is the Fastest Growing Global Brand having presence in at least 96 countries across the globe. Analytical Technologies Limited is an ISO :9001 Certified Company engaged in Designing, Manufaturing, Marketing & providing Services for the Analytical, Chromatography, Spectroscopy, Bio Technology, Bio Medical, Clinical Diagnostics, Material Science & General Laboratory Instrumentation. Analytical Technologies, India has across the Country operations with at least 4 Regional Offices, 6 Branch Offices & Service Centers. Distributors & Channel partners worldwide.

►► Our Products & Technologies



UV/VIS
Spectro 2080+
Double Beam



Infra FTIR



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Chromatograph
3007



Optima Gas
Chromatograph
2979 Plus



Flash
Chromatograph



Atomic Absorption
Spectrophotometer



Liquid Partical
Counter



Optical Emission
Spectrophotometer



DSC/TGA



Semi Auto Bio
Chemistry Analyzer



HEMA 2062
Hematology
Analyzer



Micro Plate
Reader/Washer



URINOVA 2800
Urine Analyzer



Total Organic
Carbon 3800



Fully Automated
CLIA



NOVA-2100
Chemistry Analyzer



PCR/Gradient PCR/
RTPCR



TOC
Analyzer



Laser Particle
Size Analyzer



Ion Chromatograph



Water purification
system

►► Regulatory compliances



►► Corporate Social Responsibility

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Analytical Foundation

1. Research & Innovation Scientist's awards / QC Professional Award : Quality life is possible by innovation only and the innovation is possible by research only, hence ANALYTICAL FOUNDATION is committed to identify such personalities for their contributions across various field of Science and Technology and awarding them yearly. To participate for award, send us your details of research / testing / publication at info@analyticalfoundation.org

2. Improving quality of life by offering YOGA Training courses, Work shops / Seminars etc.

3. ANALYTICAL FOUNDATION aims to DETOXYFY human minds, souls and body by means of Yoga, Meditation, Ayurveda, Health Care, Awards, Media, Events, Camps etc.

►► Reach us @



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