Experimental Nanobiotechnology

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Lecture 07: Dynamic Light Scattering and Zeta Potential Analysis

Hello everyone, today we are going to learn about dynamic light scattering and zeta potential analysis. In today's lecture, we will learn what dynamic light scattering and zeta potential are, and we will also learn about the applications of DLS analysis and the importance of zeta potential. At the end of this lecture, through practical demonstration, we will learn about DLS and zeta potential analysis. Let us see what dynamic light scattering is.

Dynamic light scattering is also called photon correlation spectroscopy. This is a technique to measure the size of particles in suspension or solution. Here, the size is determined by analyzing fluctuations in the intensity of scattered light caused by the Brownian motion of the particles. Using DLS, we can measure the hydrodynamic radius and size distribution of the particles, as well as the diffusion coefficient. Let us see the principle involved in DLS and how we can use it to measure particle size.

The first one is Brownian motion. Brownian motion is the random movement of particles in suspension due to their collision with the solvent and other molecules. Here, smaller particles diffuse faster, while larger particles diffuse more slowly. The next one is Rayleigh scattering. While the particles are in Brownian motion, they scatter light when illuminated by a laser beam.

And the scattering happens when the size of particles is smaller than the wavelength of the incident laser beam. The intensity of the scattered light fluctuates over time due to the random motion of the particles. The correlation function in the instrument analyzes the decay of fluctuation in the light intensity over time and provides information about the particle size. Let us learn about the various parts of DLS and their functions. In the DLS, we have the laser source, which provides monochromatic light.

Next, we have the attenuator, which controls the intensity of the laser beam reaching the detector. Here is the sample cell where we place our cuvette containing the samples. We

also have two detectors, and these detectors are photodiode detectors. They are used to measure the scattered light and are usually placed at 90 degrees and 173 degrees. Then we have the correlator.

The correlator analyzes the fluctuation in the intensity of the scattered light signal. All the data can be analyzed with the help of the software on the computer. Let us learn about the function of the correlator in the DLS. The correlator is the computational brain of the DLS system, which is essentially a signal comparator. It measures the fluctuation in the intensity of the scattered light at a point over a period of time.

And then correlates it to determine the diffusion coefficient of the particles. And this diffusion coefficient of particles is directly linked to their size through the Stokes-Einstein equation. The larger particles will have slower fluctuations. As you know, bigger particles move very slowly. So the larger particles will have slower diffusion and longer correlation.

Whereas the smaller particles will have rapid fluctuations. That means rapid diffusion and rapid correlation. The Stokes-Einstein equation is the theoretical basis for DLS measurements for interpreting particle size. If you want to measure the hydrodynamic radius, we have to use this equation, and the hydrodynamic diameter can be analyzed using this equation. The two equations are mentioned below

Where:
D = diffusion coefficient
k_B = Boltzmann constant
T = absolute temperature
η = solvent viscosity
r = hydrodynamic radius
d = hydrodynamic diameter

$$D = \frac{k_B T}{6\pi n r} \quad \text{or} \quad D = \frac{k_B T}{3\pi n d}$$

Hydrodynamic Radius or Hydrodynamic Diameter

Let us see what hydrodynamic size is in detail.

The hydrodynamic size represents the diameter of a hypothetical sphere that diffuses at the same rate as the particle being measured. That's why this hydrodynamic size is not the actual size of the particle because it includes the core particle size along with the molecules adsorbed on the particle surface. It may be surfactants, polymers, or ligands, and also the solvation layer, which is the layer of solvent molecules adhering to the particle due to intermolecular interactions. The hydrodynamic size is also affected by the solvent viscosity

and solvation effects. So that is why whenever we measure the size using DLS, we are measuring the hydrodynamic size.

which is slightly bigger than the size we are getting from the transmission electron microscope. The size we are getting from the transmission electron microscope is the actual particle size. For example, if the particle size is roughly around 10 nanometers, in the hydrodynamic measurement we will be getting roughly around 20 or 25 nanometers. This is because we will also have the molecules which are adsorbed on the particle surface. It may be a surfactant or it may be some of the ligands which are attached to the particle.

Let us see what the polydispersity index is. The polydispersity index (PDI) is a dimensionless number that indicates the particle size distribution in a sample. It provides insight into the distribution of uniformly sized particles within a dispersion. This PDI can be calculated based on the width of the particle size distribution relative to the mean particle size. By using this equation, we can calculate the PDI,

$$PDI = \frac{\sigma^2}{d^2}$$

Where,
σ is the width of the size distribution peak
d is the mean particle size

where sigma is the width of the size distribution peak and D is the mean particle size.

From this picture, you can see there are two peaks. This is due to the size difference in the nanoparticles. For example, the first peak is due to the nanoparticle size in the range of 10 nanometers, and the next peak is due to the nanoparticle size in the range of 100 nanometers. If you have nanoparticles of different sizes and the size distribution is very high, your PDI (Polydispersity Index) will also be high.

Let us see what PDI values are. When we synthesize nanoparticles, if you get uniform-sized nanoparticles, the PDI will be low. It will be between 0.0 and 0.1. These uniform-sized nanoparticles are mainly useful for drug delivery applications. If you have a PDI between the range of 0.1 to 0.4, that means these are moderately polydisperse systems.

That means your nanoparticles have slight variations in size. If you have a high PDI, that means above 0.4, that means the nanoparticles are aggregated, or they may be contaminated with other particles, or it may be due to poor synthesis. The particle size is bigger, and you also get a broad range of sizes. Let us see what the various factors influencing PDI are.

The first one is aggregation. When particles clump together, that leads to an increase in PDI values. The next one is sample purity. If there is contamination or any dust, that will also lead to a higher PDI value. The third one is synthesis quality.

If the synthesis protocol is not well optimized, that leads to a broad size distribution. The fourth one is measurement condition. If there is a variation in conditions like temperature or inconsistent sampling, that leads to different sizes and will affect the PDI. Here, a low PDI means it indicates high-quality, uniform-sized nanoparticles. Let us see some of the important applications of DLS analysis.

It will be very useful for nanoparticle characterization to understand the size distribution. In biotechnology, it will be useful for understanding protein aggregation and biomolecular interactions. In pharmaceuticals, it will be useful for understanding liposome-based drug release systems. In environmental science, it will be useful for analyzing colloids in water. In material science, it will be useful for studying polymer dispersions and emulsions.

In the field of cosmetics, it will be useful for optimizing pigment dyes and various emulsions. Let us see some of the advantages and limitations of DLS. The advantages are that it is a non-destructive and rapid technique and requires minimal sample preparation. Whereas the limitations are that it does not provide the actual size. It provides the hydrodynamic size, and it does not provide any information about the shape of the nanoparticle, as it assumes all particles are spherical in nature.

Let us see some of the common issues in DLS analysis and how to overcome them. The first one is high PDI. If you have a high PDI, it indicates aggregation of nanoparticles. You can try diluting the sample. The next one is multiple peaks.

If you get multiple peaks, it may be due to contaminants. You can filter the sample with a 0.22-micron filter. If you get a low signal, it may be due to insufficient particle concentration. You can increase the particle count. The fourth one is temperature fluctuation. If there is temperature fluctuation, you can use a temperature control setup. By using this, you can overcome the common issues in DLS analysis.

I hope you got an overall idea about DLS analysis. Now we move on to zeta potential analysis. Zeta potential is the electrostatic potential at the boundary between the stationary layer of fluid attached to a particle and the surrounding liquid in which the particle is suspended. Zeta potential values determine whether the particles in suspension will remain dispersed or aggregate.

If we have a high zeta potential, the stability of nanoparticles will also be high. And the zeta potential is not a property of the particle but of the system. Including the particle and the solvent. That's why if there are any changes to the medium, such as a change in the concentration of ions or a change in the pH, that will affect the zeta potential. Let us learn more about zeta potential in detail.

This is the particle, and this is the particle surface. Followed by that, we have the Stern layer, which is composed of ions tightly bound to the particle. These ions travel with the particle, and followed by that, we have the slipping plane. This is the radius within which the counterions move with the particle. Ions beyond this point do not travel with the particle, and the potential at this point is called the zeta potential. Followed by that, we have the diffuse layer. The ionic concentration in this layer is not the same as in the bulk, and an outward concentration gradient of ions exists until it equilibrates with the bulk.

Let us see the principle of zeta potential measurement. The main principle behind zeta potential measurement is electrophoresis, which is the migration of molecules and particles due to the presence of an electric field. To measure zeta potential, a technique called electrophoretic light scattering is utilized. The working principle is: the sample is illuminated by a laser beam, and when an electric field is applied, the charged particles move toward the oppositely charged pole and attain a certain velocity. The movement of particles causes a Doppler shift in the frequency of the light scattered by the particle.

You can see here that if you have negatively charged particles, these particles move towards the positive charge. When the particles are moving, that will cause the Doppler shift, and that will lead to a difference in the frequency of the light scattered by the particles. The changes in frequency from the Doppler shift are used to calculate the velocity of the particles using this equation.

$$\frac{\Delta f}{f} \propto \frac{n_0 v}{c}$$

where: c =speed of light in vacuum.
n_0 =solvent refractive index.
f = frequency of the incident light

Then, electrophoretic mobility is calculated, which is related to the velocity of the particles,

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

where: μ_E = electrophoretic mobility
v = electrophoretic velocity of particles
E = applied electric field

and can be done by using this equation.

Finally, by using this equation, we can calculate the zeta potential, which relates the electrophoretic mobility to the zeta potential.

$$\zeta = \frac{\eta \mu_E}{\varepsilon_r \varepsilon_0}$$

where: ζ = zeta potential
η = viscosity of the solution
μ_E = electrophoretic mobility
ε_r = solvent dielectric constant
ε_0 = vacuum permittivity

Let us learn about zeta potential and particle stability. The magnitude of zeta potential gives an indication of the stability of the colloidal system. If all the particles have a large zeta potential, they will repel each other, leading to dispersion stability. It means if you have particles with a uniform charge and if you have a high charge, then these particles repel each other. That means you have a large zeta potential. If the particles have low zeta potential values, then the repulsion force disappears, and particles start aggregating, leading to dispersion instability.

So if you have a low charge, the particles will try to come closer and aggregate. And the dividing line between the stable and unstable aqueous dispersion is generally taken at +30 or -30 millivolts. From this table, you can understand that if you have a particle with zeta potential between 0 to + or -10, it means it is highly unstable. If you have + or -10 to 20, this is limited stability. If it is between + or -20 to 30, it is moderately stable, and if it is more than + or -30, this is highly stable.

Let us see the role of pH in zeta potential. The pH of the suspension plays an important role in the stability of the particles. Changes in the pH influence the surface ionization of

the functional groups. And most of you know this isoelectric point. The isoelectric point is the pH where the zeta potential equals zero.

If you have a low pH, that means positive charge dominates on the surface, and you get a positive zeta potential. And if you use a high pH, negative charge dominates on the surface, and you will get a negative zeta potential. At the isoelectric point, you have zero charge. So due to zero charge, you get zero zeta potential, which leads to particle aggregation. Let us see the importance of zeta potential.

The zeta potential is useful in determining the stability of particles in a colloidal system, and in drug formulation, it helps in optimizing the emulsion parameters for better drug stability. It can also be used to study surface functionalization by analyzing changes in the zeta potential. For example, we have a nanoparticle, and if we use PEG, that is polyethylene glycol, as a capping agent, it will give a negative charge, and if we use PEI, that is polyethyleneimine, it gives a positive charge to the nanoparticle.

So by using this zeta potential analysis, we can also understand the surface functionalization and the effect of pH, and the dispersion stability can also be studied by using this zeta potential. And this zeta potential is not only useful for nanotechnology and nanomaterials, but it also helps in the manufacturing process of paints, pigments, and cosmetics. Let us see some of the common issues in zeta potential analysis and how to overcome them. The first one is poor sample preparation. If the sample contains impurities or dust, that can be removed by filtering the samples using suitable syringe filters, and make sure that the sample is homogeneously dispersed.

You can use ultrasonication if required and ensure proper dilution concentration within the instrument range. And it may also be due to incorrect pH or ionic strength. If there is incorrect pH or ionic strength, that can shift the zeta potential unpredictably. So adjust the pH carefully using calibrated buffers or acids and bases. The third one is electrode and cuvette contamination, which can lead to unreliable results.

So, it is always better to wash the cuvettes with distilled water or you can use fresh disposable cuvettes. The fourth one is aggregation of particles, which can alter the zeta potential values. For that, stabilize the sample with appropriate surfactants and avoid sample preparation conditions that approach the isoelectric point. So as I mentioned earlier, if the sample is at the isoelectric point, then it tends to aggregate.

The fifth one is temperature fluctuation, which can change the particle mobility and medium viscosity, altering the zeta potential. So, it is always better to maintain a constant temperature during the measurements and also record and report the measurement temperature for consistency. I hope you understood the basics and principles of DLS and zeta potential analysis. Let us go to the lab and learn this technique in more detail. In this experiment, we will learn how to perform zeta potential and hydrodynamic size measurements of nanomaterials.

In today's experiment, we will measure the zeta potential and hydrodynamic size of tannic acid-capped gold nanoparticles, which we synthesized earlier in one of the previous lab demonstrations. To proceed with the experiment, the materials required are a zeta-sizer cuvette, a four-sided DLS cuvette, microtips, micropipettes, ultra-pure water, tissue paper, and a wash bottle with ultra-pure water in it. First, we will measure the zeta potential of the synthesized gold nanoparticles. Let us start by taking the zeta-sizer cuvette and washing it thoroughly. Dry the cuvette using tissue paper.

Next, carefully add the sample into the cuvette, ensuring the liquid fills up to the copper electrodes. The sample must touch both electrodes. Now, turn on the machine. It will warm up, and you should wait until the indicator light turns green. Then, open the Zetasizer software on the system.

Before starting the experiment, adjust the settings and set the parameters. Choose the measurement type as Zeta potential. Enter the sample name. Select the cuvette material as polystyrene latex. For the dispersant, we have used ultra pure water.

So here we have to choose water. In the general options, select the appropriate model. In the temperature settings, set the equilibrium time to 120 seconds. Choose the cell code corresponding to the cuvette. In the measurement settings, select automatic so that the machine will conduct the runs automatically based upon the type of sample.

Click OK in the data processing section. Once all settings are configured, place the cuvette inside the machine. Open the lid. Insert the cuvette and close the lid. Press start.

The machine will equilibrate for 120 seconds. Then, the machine will optimize before beginning to measure the sample. The machine will take triplicate measurements, with the number of runs per measurement varying depending on the sample. This process will be handled automatically by the machine, as we have selected the automatic measurement setting. Here, you can see the data from three measurements.

We have to select all together and press the zeta potential button. You will see the data from the three measurements displayed, each shown in a different color. The zeta potential of the tannic acid-capped gold nanoparticle is 20.1 millivolts, with a standard deviation of 5.01. Finally, save the data. Now, remove the cuvette from the machine and wash it with ultrapure water.

Next, we will proceed with the hydrodynamic size measurement using dynamic light scattering (DLS). Open the same software. This time, select 'Size' as the measurement type. Enter the sample name. The remaining parameters are the same, except for the cell.

Since we are using a different cuvette, make sure to select the correct one in the software. Once the parameters are set, add the sample to the four-sided cuvette and place it in the machine. Press start in the software to begin the experiment. Similar to the zeta potential measurement, the machine will equilibrate for 120 seconds, then optimize before starting the measurements. The machine will take triplicate measurements

and the number of runs per measurement will vary based on the sample. As I told earlier, the machine will conduct the measurements automatically based on the type of the sample. Once the measurements are complete, you will see the data from the three measurements displayed. Choose the data together and press intensity to view the graph. The average particle size of the tannic acid cap gold nanoparticle is approximately 110.4 nanometer.

Finally, save the data from the DLS size measurement. Save the data. So in this experiment, we have learnt how to perform zeta potential and hydrodynamic size measurements of tannic acid cap gold nanoparticles. As a summary, in today's lecture, we learnt about DLS and zeta potential analysis and parts of the instrument and principle. Also, we learnt how to perform DLS and zeta potential measurements through practical demonstration.

Thank you for your kind attention. I will see you in another interesting lecture.