Particle Size Studies for Biotechnology and Life Science

- Application Proposal from HORIBA Analytical Solution Plaza -

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Particle size analyzers are used for R&D and quality control purposes in a wide range of fields, including ceramics, battery materials, catalysts, pigments, cosmetics, foods, and pharmaceuticals. In various industrial processes, the size of particles, including powders, is an important factor in characterizing the functionality of products. The application of particle size analyzers has been remarkably expanding not only in the field of nanotechnology, but also in the fields of biotechnology and life sciences. Particularly in the process of drug development and manufacturing, drug modalities are diversifying, including antibody drugs, nucleic acid drugs, cellular drugs, and gene therapy, as well as small molecule drugs, which have been the mainstream in the past and there is a growing need to evaluate cell-derived products such as exosomes, liposomes and drug delivery systems (DDS). Biopharmaceuticals have more complex molecular structures and must be analyzed and evaluated while considering the degree of damage to the sample. Therefore, conventional analytical principles and methods may not be applicable and different analytical approaches need to be considered. For small molecule pharmaceuticals as well, there are increasing demands for evaluation using different scales, rapid testing methods, high throughput and it is essential to propose analyzers and applications that satisfy these demands.
The HORIBA Group possesses multiple particle size analysis technologies that are suited to different measurement targets and offers a wide range of solutions. This paper focuses on dynamic light scattering technology, which has been gathering attention in recent years among particle size analysis technologies and introduces its applications in the fields of biotechnology and life science.

Introduction

In the design of pharmaceutical products that affect the human body, various studies have been conducted to ensure efficacy and safety, to develop tablets that are easy to take, and to develop DDS (Drug Delivery System) that aims at more efficient drug delivery. Particle size is one of the most important design factors, and there are a wide variety of evaluation methods. To evaluate micron-sized particles that are common as raw materials and excipients for low-molecular-weight drugs, a number of measurement methods have been developed to suit the supply format, such as wet measurement methods in which particles are dispersed in water or organic solvents, dry measurement methods in which granulated powders are measured as powders, and other methods in which spray samples are measured. Particle size analyzers for this size range are commonly based on laser diffraction and scattering. On the other hand, for particle size evaluation of nano-sized particles such as polymers and biopharmaceuticals, instruments based on the dynamic light scattering principle are effective.

As shown in Figure 1, HORIBA offers various applications in the fields of biotechnology and life science. This

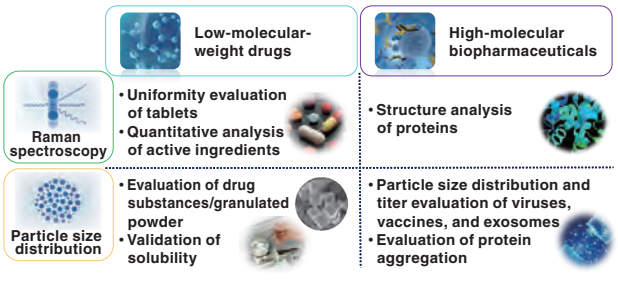


Figure 1 Analytical cases for small molecules and biopharmaceuticals.

Figure 2 Equipment nanoPartica SZ-100V2.

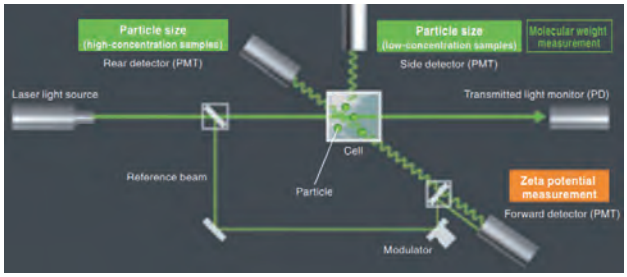
paper introduces examples of particle size distribution measurements for the biotechnology and life science fields, with a focus on products using particle size technology.

Dynamic Light Scattering Technology

Dynamic Light Scattering (DLS)

Particles are in Brownian motion in the liquid phase and when these particles are irradiated by laser light, the laser light is scattered to various angles. By following the time variation of the scattered light intensity at a fixed point from a certain angle, we can observe the fluctuation of the scattered light intensity associated with the Brownian motion of the particles. Since small particles move fast and large particles move slow, the scattered light intensity fluctuates at a high frequency for small particles and at a low frequency for large particles. From the fluctuation signal of the scattered light intensity, an autocorrelation function is calculated, and the diffusion coefficient is calculated by processing the autocorrelation function. From the diffusion coefficient, the particle size can be determined using the Stokes-Einstein equation. This is the principle of DLS (Figure 3).

Analyzers using DLS can also measure zeta potential using the laser Doppler method and molecular weight from light scattering intensity using Debye plots.



Stokes-Einstein equation $d = \frac{k_B T}{3\pi\eta D}$

d: particle size, k_B: Boltzmann constant, T: absolute temperature, η: viscosity, D: diffusion coefficient

Figure 3 Optical system of nano partica SZ-100V2.

Figure 4 Gel unit.

Recently, there is also a gel unit option (Figure 4) that enables gel analysis. Similar to the fluctuation of scattered light due to the Brownian motion of particles, gels also have fluctuations in the scattered light. Because gels contain solvent in their mesh structure and their structure is fluctuating, the scattered light obtained by irradiation of laser light can have fluctuation information. In many cases, the mesh structure of a gel differs depending on the position, so a single point measurement in a homogeneous system, such as particle size, cannot represent the entire gel. Therefore, it is necessary to measure at multiple points and get the average.

Particle Tracking Analysis (PTA)

In PTA, a camera is used to observe the bright spots of particles in Brownian motion. Since the particle size distribution is obtained by counting particles one by one, the vertical axis is an absolute value, and the greatest feature of PTA is that it provides information on the number concentration of particles. The principle is to irradiate a laser beam onto particles in Brownian motion in the liquid phase, observe the light scattered by the particles on the CMOS camera screen, and track the movement of individual particles (Figure 6). The diffusion velocity is calculated from the distance traveled by the scattered light, and the diffusion velocity is converted to the particle diameter by the Stokes-Einstein equation, which enables the particle size distribution to be obtained. The number concentration can also be calculated from the number of

Figure 5 ViewSizer3000.

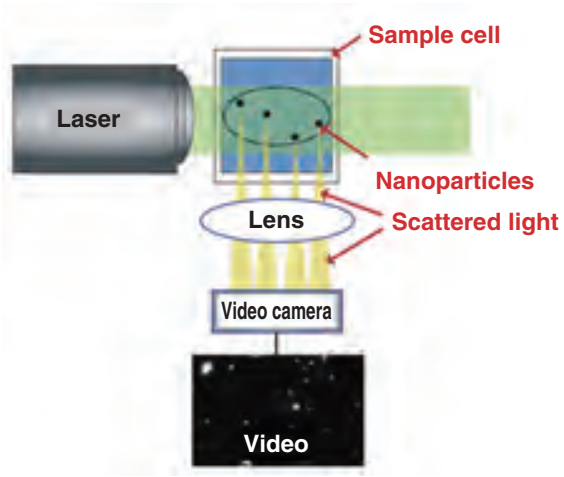


Figure 6 Instrument configuration of ViewSizer3000.

scattering centers captured on the camera screen and the volume of the measurement section. Furthermore, if particles have fluorescent characteristics, they can be separated from the scattered light using a fluorescent filter, allowing measurement of particles with fluorescent characteristics separately.

The PTA-based ViewSizer 3000 is equipped with a laser light source with three wavelengths, enabling simultaneous analysis of a wide range of particle sizes, from small to large, and it particularly suited to measure polydisperse samples. It is also possible to measure fluorescently labeled samples by using fluorescent materials that match the respective wavelengths. Furthermore, the sample cell design that includes periodic mixing of the sample ensures high reproducibility.

Application Examples

Example of exosome analysis

Exosomes are one of the extracellular vesicles ejected from cells. Exosomes contain functional factors such as membrane proteins and miRNAs derived from the ejected cells, and since exosomes can provide information on cell behavior, they are attracting attention in regenerative and preventive medicine, such as biomarker and drug efficacy research. Research is also being conducted to analyze the functional factors expressed by exosomes, one of which is a technology for fluorescent labeling and discrimination of specific functional factors. PTA can discriminate particles with fluorescent characteristics and determine their number concentration and particle size distribution. In this paper, we will describe an example of fluorescent labeling of CD9, a membrane protein contained in exosomes, and evaluation of the number concentration and particle size distribution using PTA.

First, the size distribution and number concentration of all particles of exosomes derived from human serum were measured. Next, particles fluorescently labeled with Alexa 488 for CD9, a membrane protein of human serum-derived exosomes, were viewed on the camera screen, and particle diameter and particle number concentration were measured in the fluorescence measurement mode (fluorescence only was observed by using a long-pass filter (LPF) to cut off scattered light). The number concentration of total particles was 2.3×10^8 counts/mL and that of fluorescent particles was 5.5×10^7 counts/mL, indicating that approximately 24% of exosomes expressed CD9. (Figure 7 and Figure 8)

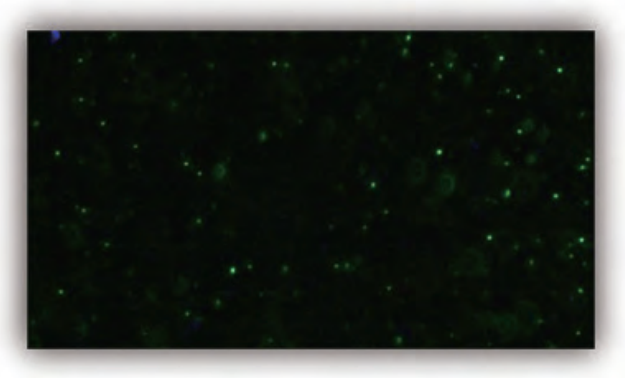


Figure 7 Exosome whole particle image by ViewSizer3000.

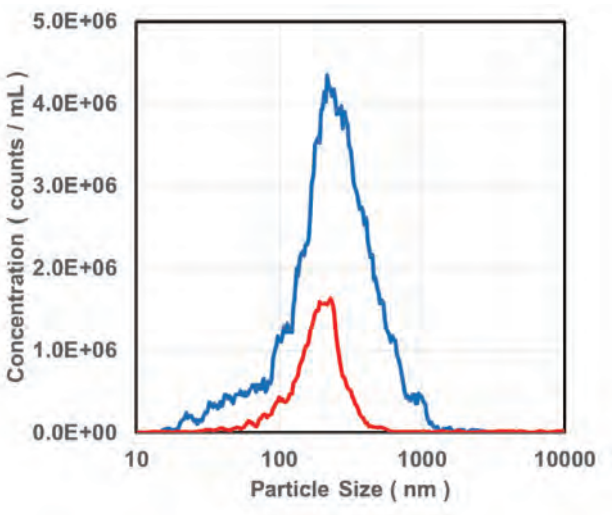


Figure 8 Comparison of ViewSizer3000 results between exosome whole particles and fluorescently labeled particles.
— : Exosome whole particles
— : Fluorescence labeled particles.

Example of liposome analysis

Many factors that determine whether a drug treatment is successful or not remain unknown, partly because there is no way to confirm that the drug has reached the target tumor tissue. In recent years, research on drug delivery systems (DDS) has progressed, and a promising approach utilizing liposome has been developed. This innovation relies on the property of liposomes to form a closed lipid vesicle structure encapsulating an aqueous phase inside the lipid bilayer membrane. Many "liposomal drugs" are already on the market. On the other hand, focusing on the lipid bilayer, which is a component of liposomes, it is possible to reconstitute proteins, etc. in the lipid bilayer and therefore, it is thought that the use of photosensitizers, etc. will make it possible to confirm that the target tumor tissue is reached. Therefore, liposomes are expected to be a useful tool as "theranostics" that has both therapeutic (Therapy) and diagnostic (Diagnostics) functions (Figure 9). In DDS, the particle size of liposomes should be designed to be 20 to 200 nm, taking into account the EPR effect* in tumors (Figure 10).

In this paper, we present a case study of liposomes as a theranostics formulation, in which we investigated the effect of photosensitizer content in the lipid bilayer on the particle size of the liposomes.

Liposomes were loaded with photosensitizers in various proportions and the particle diameters of the liposomes at various content levels (0.0, 0.5, 1.0, 5.0, and 10.0%) were measured using a dynamic light scattering analyzer nanoPartica SZ-100V2. The results showed that the particle size distribution of liposomes (Figure 11) became broad and the arithmetic mean diameter increased with increasing photosensitizer content. In both cases, the EPR effect was confirmed to be within the range of the EPR effect.

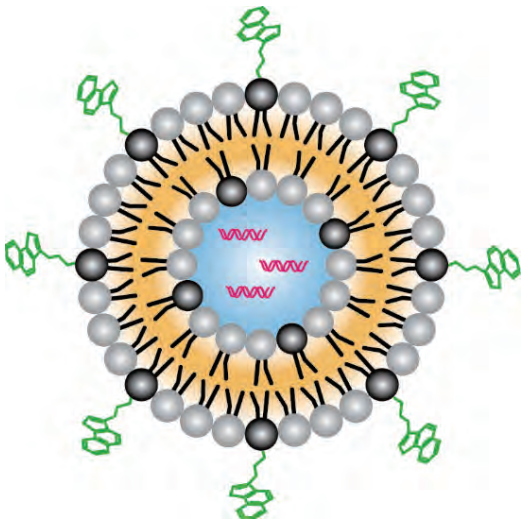


Figure 9 Theranostics Liposomes.

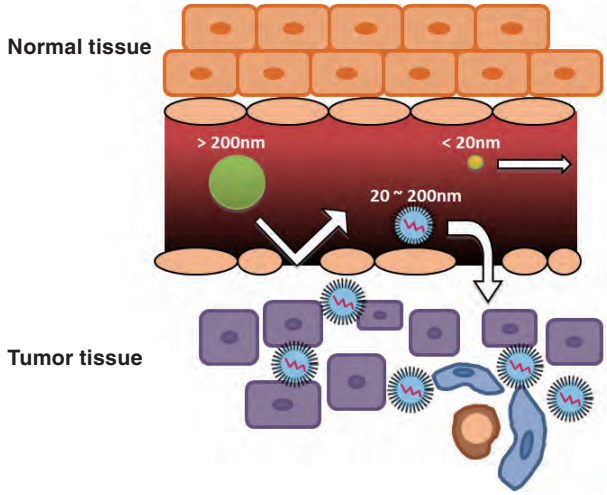


Figure 10 EPR effect.

* EPR effect: The vascular endothelium of tumor tissue has a coarser crevice structure than that of normal tissue, which allows particles of a specific size (20-200 nm) to penetrate more easily.

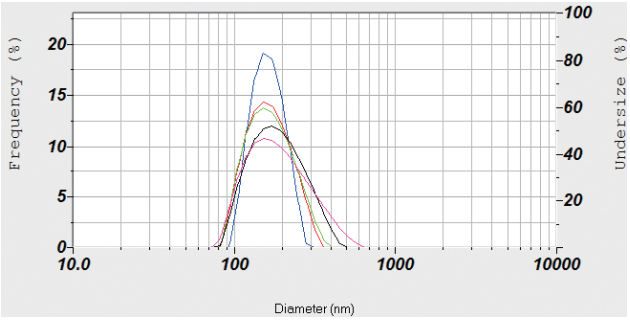


Figure 11 Particle size distribution depending on surface drug content.
— : 0.0% — : 0.5% — : 1.0% — : 5.0% — : 10.0%

The dynamic light scattering measurements provide insight into the "photosensitizer content that reconstitutes the lipid bilayer" and the "particle size of liposomes" (related to the EPR effect). Combining these results with those obtained from zeta potential and fluorescence measurements, we can more accurately evaluate the diagnostic function of liposomes as theranostics.

Example of Analysis of Cellulose Nanofiber

Cellulose nanofiber (CNF) is a fibrous material made mainly from cellulose obtained from biomass such as wood and pulp, and has many characteristics such as lightness, high strength, high transparency, high elastic modulus, low linear thermal expansion coefficient, and large specific surface area. Because of its low environmental impact, its use has been studied in various fields, including automobiles, home appliances, housing and building materials, cosmetics, and life sciences and its

practical application is also progressing. CNF is classified as a physical gel, and is physically entangled, and the entangling force includes not only three-dimensional entanglement but also electrostatic force. The mesh structure collapses when force is applied. Therefore, at present, only rheological and other evaluations are available, and the analysis of mesh structure using nanoPartica SZ-100V2 gel units, which can be evaluated in terms of distribution and numerical values, is attracting attention. As mentioned above, gels have a non-uniform mesh structure from place to place, so it is necessary to change the measurement points and average the results. The gel unit, developed as an option for the Dynamic Light Scattering Particle Size Distribution Analyzer, automatically operates the measuring cell in the vertical direction, enabling measurement at multiple points.

The following is an example of analysis of CNF with different cellulose concentrations using this gel unit.

Samples with cellulose concentrations of 0.2%, 0.5%, and 1.0% were measured (Figure 12). For each sample, the autocorrelation function was measured at 10 different measurement points of 100 µm each and the averaged results are shown in Figure 13. It was confirmed that the higher the cellulose concentration, the smaller the mesh size became.

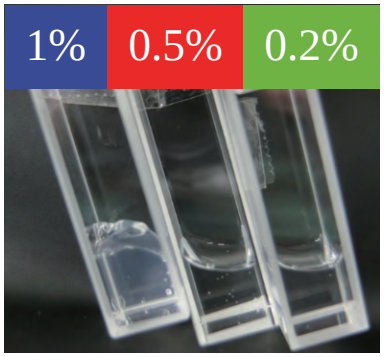


Figure 12 Samples with various cellulose concentrations.

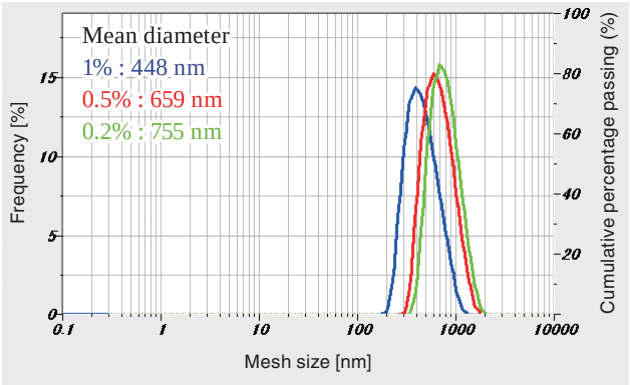


Figure 13 Mesh size distribution at various concentrations of CNF.

As described above, we expect to contribute to the fields of biotechnology and life science, including applications for exosomes, liposomes, and CNFs, and we intend to expand the scope of our efforts so that we can offer proposals for various modalities by utilizing the features of the various products in HORIBA's product lineup. We look forward to expanding our efforts to offer solutions for various modalities by taking advantage of the features of HORIBA's product lineup.

Conclusion

In this paper, we focused on particle size distribution, especially dynamic light scattering in the biotechnology and life science fields, and introduced some examples of analysis along with their measurement systems. We have shown that dynamic light scattering can contribute from various angles, such as evaluation using a different scale, establishment of rapid testing methods, and high-throughput, by being used for analysis of various modalities as well as conventionally used low-molecular-weight pharmaceuticals.

In addition to dynamic light scattering technology, particle size distribution is also contributing to various research and development, such as the analysis of fluorescent bacteria using laser diffraction/scattering particle size technology. The HORIBA Group is constantly working to develop analytical measurement technology and to propose analytical methods. We will work together with our customers on the issues they face and strive to provide total solutions on which they can rely on our experience.

* Editorial note: This content in based on HORIBA's investigation at the year of issue unless otherwise stated.

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