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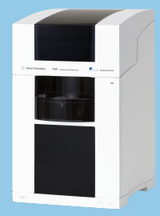
Application Data

Bio·Pharmaceuticals

ELSZ-2000

Zeta-potential &
Particle size Analyzer



Agilent 7100

Capillary Electrophoresis

FDLS-3000

Fiber-Optics Dynamic Light Scattering
Spectrophotometer

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Bio • Pharmaceuticals

ELSZ

Zeta-potential &
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CE

Capillary
Electrophoresis
Agilent 7100

FDLS

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Proteins, enzymes, peptides, and nucleic acids

Pharmaceuticals

Small molecule pharmaceuticals

Polymer pharmaceuticals

DDS and others

1 FDLS Particle diameter evaluation of chaperonin, a protein that aids in the folding of other proteins

Purpose Proteins consist of polypeptides, or chained amino acids. Polypeptides are folded to form unique steric structure and generate functions unique to proteins. Proteins were previously believed to fold spontaneously, since their steric structures are determined by the arrangement of amino acids configuring the proteins. In recent years, however, researchers have come to realize that other helper proteins are required for an individual protein to assume its unique steric structure. These proteins are called molecular chaperones. Among them is a specific protein known as chaperonin. Chaperonin consists of two subunits, GroEL and GroES. GroEL bonds to polypeptides to be folded, while GroES covers GroEL and controls the action of GroEL.

Result We used the FDLS-3000 fiber optic dynamic light scattering spectrophotometer to measure hydrodynamic radius of the GroEL of chaperonin by the dynamic light scattering method. The particle diameter determined from scattering coefficient proved to be 17.9 nm. The relationship between the hydrodynamic radius and the radius of rotation is known to be given by the equation Rg = (3/5)1/2Rh. If we enter Rh x 2 = 17.9 nm in the equation to calculate Rg, the result is Rg = 6.9 nm. As determined by X-ray small angle scattering measurement, Rg is 6 to 7 nm, a value that agrees well with the results given by dynamic light scattering analysis. Measurements with FDLS-3000 appear to give suitable results.

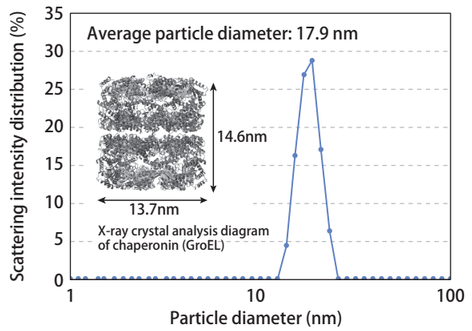


Fig. 1 Distribution of particle diameters of molecular chaperonin (GroEL)
Data source: Professor Yasushi Kawata, Department of Engineering, Tottori University

2 ELSZ Separation analysis of protein aggregates using an ultracentrifuge

Particle diameter

Purpose Ultra-centrifugal force fields are used as a research tool for polymer solutions. One such instrument is the analytical ultracentrifuge used for molecular weight measurements based on sedimentation equilibrium and sedimentation coefficient measurements; another is the separation ultracentrifuge used to fractionate organic matter.

We used an ultracentrifuge to remove aggregates of BSA (bovine serum albumin) to separate them entirely into monomers.

Result BSA was dissolved in 10 mM aqueous NaCl solution to concentration of 1 mg/ml. After centrifugation at 150,000 rpm for 30 minutes using the solution and ultracentrifuge, we measured the diameter of the particles in the solution. Immediately after dissolution, we confirmed the presence of aggregates based on observed peaks at 100 nm or above and peaks at 10 nm or below. Based on a single observed peak at 10 nm or below, centrifugation resulted in a solution composed almost entirely of monomers.

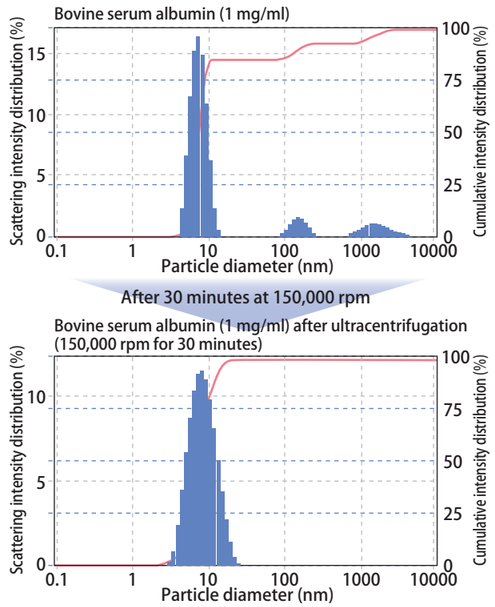


Fig. 1 Distribution of particle diameters of BSA solution before and after centrifugation (upper: before centrifugation; lower: after centrifugation at 150,000 rpm for 30 minutes)

3 ELSZ Particle diameter evaluation of proteins of different molecular weights

Particle diameter

Purpose Formed by the integration of nanotechnology and biotechnology, nanobiotechnology is a new field that seeks to elucidate life phenomena using nano-level analysis technology and to apply the results to the fields of pharmaceuticals and electronics materials. Biopolymers such as DNA and proteins have particle diameters ranging from several to tens of nanometers. Essentially, these are nanomachines that control the phenomena associated with life. Evaluations of their physical properties often entail measurements of particle diameter, often using a dynamic light scattering method capable of measurement of several nm level.

We dissolved proteins of varying molecular weights—namely, bovine serum albumin (BSA, monomer Mw = 66,000), ovalbumin (OVA, Mw = 45,000), and lysozyme (Mw = 14,000)— in a 10 mM phosphate buffer solution (pH7) to achieve a concentration of 1 mg/ml and performed measurements after optical purification (filtration through a filter with a pore size of 0.1 μm).

Result Figure 1 shows the measurement results. The average particle diameter of BSA is 8.5 nm, consistent with data from the literature (7 to 9 nm). The primary particle diameters of OVA and lysozyme with molecular weights smaller than that of BSA were determined to be 7.8 nm and 3.8 nm. The peak corresponding to large particles found in the distribution of particle diameters of lysozyme is believed to be due to dust not removed by filtration. Measurement of proteins of small particle diameters tends to be affected by dust because scattering intensity is weaker compared with substances of larger particle diameter.

Thus, filtration is an important preliminary step when measuring protein particle diameters of several nanometers.

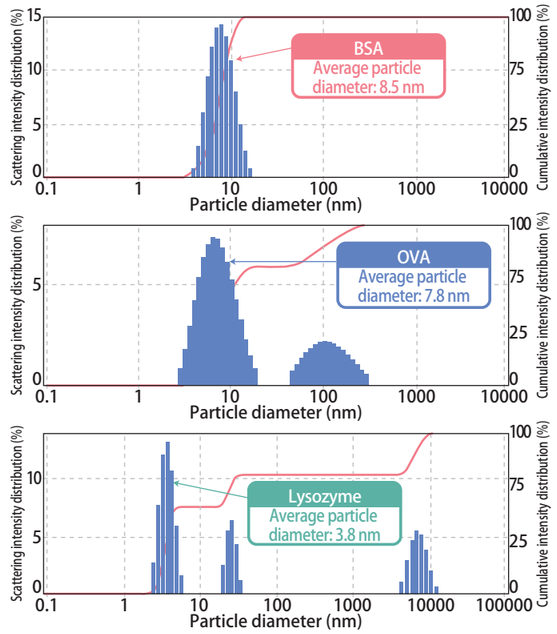


Fig. 1 Distribution of particle diameters of BSA, OVA, and lysozyme

4 ELSZ Evaluating the scattering coefficient of various proteins by dynamic light scattering

Particle diameter

Purpose The scattering coefficient of proteins depends on molecular weight and the shape of the protein molecules. Although scattering coefficient of particles can be determined by various methods, dynamic light scattering method determines scattering coefficient of particles in solution most easily and enables calculation of hydrodynamic radius using the Stokes-Einstein equation. Molecular weight can also be estimated based on a given scattering coefficient.

We determined the scattering coefficient of substances (lysozyme, trypsin inhibitor, anhydrase, ovalbumin, and bovine serum albumin) of molecular weights ranging from 10,000 to 70,000 and obtained correlations with molecular weights.

Result Table 1 gives the scattering coefficients of proteins of different molecular weights. Following equation gives the relationship between molecular weight (Mw) and scattering coefficient (D):

$$D = K_D \times Mw^{-\alpha D}$$

A double-logarithmic plot of molecular weight and the scattering coefficient shows a linear relationship. We can obtain K_D and αD from the slope and the intercept (Fig. 2).
We can calculate the molecular weight of an unknown protein based on the scattering coefficient using the equation given below:

$$D = 6.473 \times 10^{-5} \times Mw^{0.4324}$$

Table 1 Result of measurements of scattering coefficients of proteins of different molecular weights (solvent: 10 mM phosphate buffer solution (pH7); concentration: 2 mg/ml)

Protein	Molecular weight	Scattering coefficient (cm^2/s)
Lysozyme	14000	1.05×10^{-6}
Trypsin inhibitor	20100	8.48×10^{-7}
Anhydrase	30000	7.86×10^{-7}
Ovalbumin	43000	6.65×10^{-7}
Bovine serum albumin	66000	5.15×10^{-7}

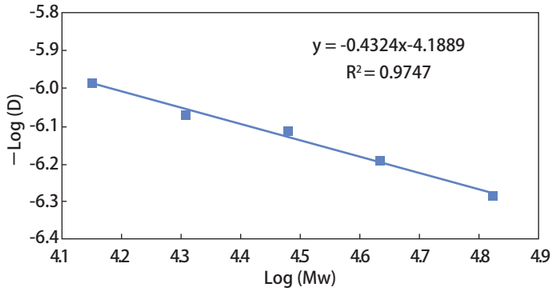


Fig. 1 Double-logarithmic plot of scattering coefficient (D) and molecular weight (mM)

5 ELSZ Detection of aggregates of proteins by dynamic light scattering

Particle diameter

Purpose In biopharmaceuticals, dimers in peptide-protein aggregates are strictly regulated by size exclusion chromatography. However, protein aggregates of 0.1 µm to 10 µm are not regulated. This presents a crucial problem in light of the immunogenicity risk of proteins.

We measured the particle diameters of proteins using dynamic light scattering.

Result The figure shows the result of particle diameter measurements of enzyme proteins of molecular weights around 20,000. The results showed two peaks, one at 4.8 nm and one at 57.1 nm. Based on size, particles of diameters measuring 4.8 nm were determined to be monomers.

Particles of 57.1 nm are believed to be denatured aggregates.

Since it depends on molecular weight and size, light scattering measurement is an effective way to detect protein aggregates.

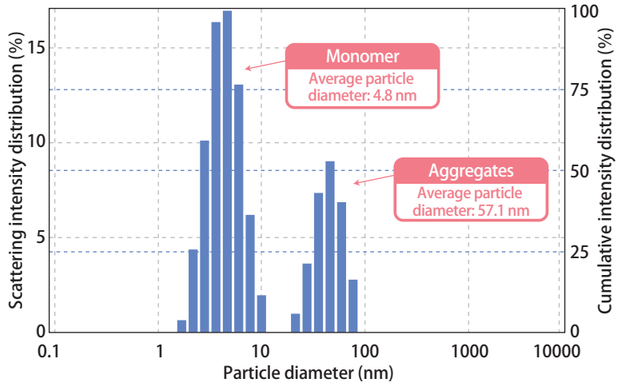


Fig. 1 Distribution of particle diameters of enzyme proteins

6 ELSZ Zeta potential evaluation of acidic proteins

Zeta potential

Purpose Protein structure and function are determined by amino acid sequences. Amino acid sequences also determine protein charge states.

We measured the electrophoretic mobility of acidic proteins using an electrophoretic light scattering photometer to obtain zeta potential. The proteins examined were bovine serum albumin (monomer, Mw = 66,000), ovalbumin (Mw = 45,000), and β-lactoglobulin B (Mw = 18,000) obtained from Sigma-Aldrich. We prepared samples using a 10 mM phosphate buffer solution (pH7) at protein concentration of 1 mg/ml and filtrated them through a 0.1 µm filter. Measurements were made at 25°C and zeta potential calculated using the Smoluchowski equation.

Result Figure 1 shows electro-osmosis plots of bovine serum albumin. Table 1 shows the result of measurements of each sample.

The zeta potential of each protein was found to have a negative charge. Since these proteins are acidic proteins (i.e., the isoelectric point lies on the acid side), the values may be appropriate.

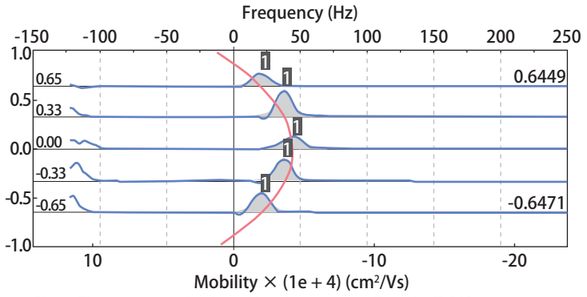


Fig. 1 Electro-osmosis plot of bovine serum albumin (BSA)

Table 1 Electrophoretic mobility and zeta potential of each protein

	Electrophoretic mobility (10^{-4} × cm²/vs)	Zeta potential (mV)
Bovine serum albumin	-1.983±0.247	-25.3±1.0
Ovalbumin	-2.309±0.137	-29.6±1.7
β-lactoglobulin B	-1.983±0.247	-25.3±3.2

7 ELSZ Heat transfer analysis of bovine serum albumin by dynamic light scattering

Particle diameter Zeta potential

Purpose The factors that denature proteins include heat (high temperature and freezing at low temperature), exposure to acids or bases, and chemical change by a denaturant. The heat denaturation of proteins is a phase or heat transition; the phenomenon has important biological implications.

We measured particle diameter and zeta potential in the context of phase transitions of bovine serum albumin (BSA) induced by heat by the light scattering method.

Result Figure 1 shows temperature dependence of average particle diameter of BSA.

We found that average particle diameter of BSA increased stepwise from 60°C to 90°C, indicating the progress of aggregation/gelation. This is consistent with the results of Raman spectroscopy.[1] Further, the gelled BSA was found to be irreversible. Figure 2 shows the temperature dependence of zeta potential of BSA. Zeta potential also increases the negative charge as temperature rises, demonstrating good agreement with change in particle diameter. This is considered to be due to the exposure of dissociable groups induced by structural changes.

1) V.J.Lin and J.L.Koenig Biopolymers, 15, 203 (1976)

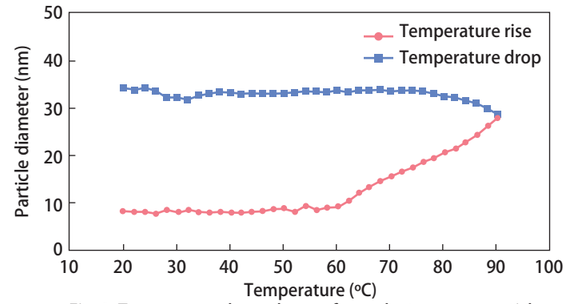


Fig. 1 Temperature dependence of cumulant average particle diameter of BSA

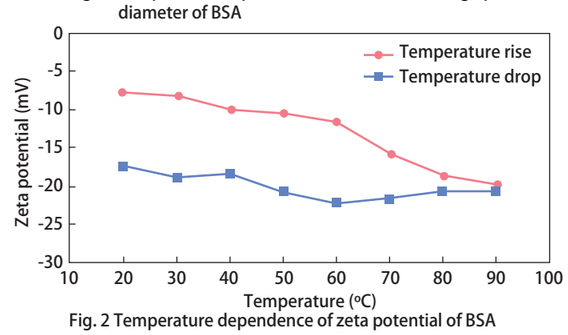


Fig. 2 Temperature dependence of zeta potential of BSA

8 ELSZ Zeta potential evaluations of proteins using pH titration

Zeta potential

Purpose Evaluating the zeta potential of proteins at each pH is important in controlling the dispersion/cohesion and adsorption to surfaces of solids such as test containers. Proteins are known to exhibit different isoelectric points depending on the pKa of amino acids forming the proteins.

We used bovine serum albumin (BSA), a protein containing higher ratios of neutral amino acids, and lysozyme, a protein containing higher ratios of basic amino acids, to measure the pH dependence of zeta potential of each.

Result BSA and lysozyme were each dispersed in 10 mM aqueous sodium chloride solution and the zeta potential was measured at each pH.

Figure 1 shows the results. The isoelectric point of BSA is pH = 4.9, while that of lysozyme is pH = 9.7.

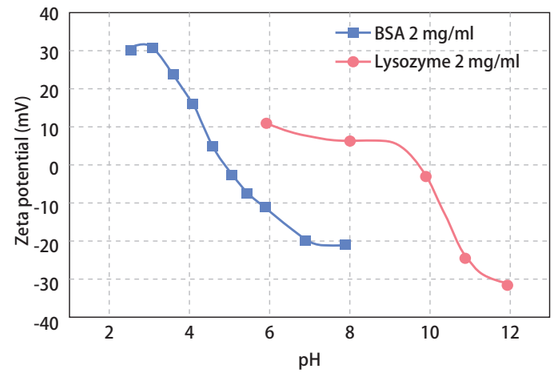


Fig. 1 Result of pH titration measurement of BSA and lysozyme

Data source: Dr. Nahoko Miyake, Tokyo Dental College

Proteins, enzymes, peptides, and nucleic acids

9 CE Analysis of basic proteins by free zone electrophoresis

Separation analysis

Purpose In analyses of basic proteins by capillary electrophoresis, if the pH of the carrier solution is lower than the isoelectric point (pI) of the protein, positively charged proteins tend to be adsorbed to the negatively charged inner surface of capillaries.

This makes analysis more difficult due to the broader shape of the peaks of basic proteins.

We suppressed the adsorption of basic proteins by treating the inner surface of the capillary with cationic polymer (within one hour required for preparation) to carry out analysis. We used bovine serum albumin (pI = 4.7) as an acidic protein, and both ribonuclease A (pI = 9.3) and lysozyme (pI = 11) as a basic protein.

We used a Tris-HCl buffer solution adjusted to pH7.0 as the carrier solution.

Result Figure 1 shows the results of using a capillary treated by a cationic polymer.

At pH7, the positively charged lysozyme and ribonuclease A were unaffected by adsorption, allowing analysis.

Since this treated capillary can also be used for negatively charged BSA, we found that this had applications to the separation of various proteins with a wide range of isoelectric points.

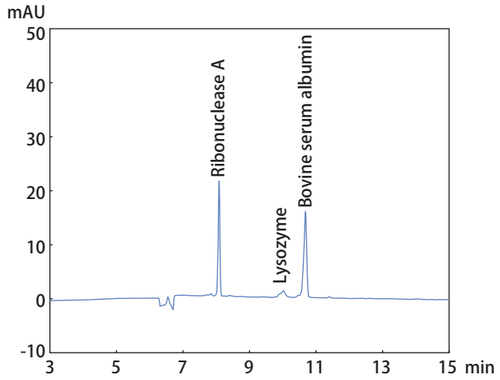


Fig. 1 Electropherogram of bovine serum albumin, ribonuclease A, and lysozyme (50 ppm each)

10 CE Analysis of proteins with molecular sieve in the presence of SDS

Separation analysis

Purpose SDS (sodium dodecyl sulphate)-polyacrylamide gel electrophoresis (SDS-PAGE) is a protein separation method. This method involves separation based on differences in molecular weight after denaturing the higher-order structures of the target proteins. It is widely used because it allows the confirmation of molecular weight and impurities.

In recent years, solutions of some polymers were found to have molecular sieve effects that are comparable to the effects of gel. Such molecular sieve is also useful for capillary electrophoresis.

We performed measurements with proteins of molecular weights ranging from 1,400 to 98,000.

Result Figure 1 shows measurement result for six kinds of proteins of different molecular weight. Proteins of smaller molecular weight are more rapidly detected.

Additionally, the linearity of the plots of the reciprocal of detection times and the logarithm of the molecular weight in Figure 2 clearly shows that separation is achieved with the molecular sieve. This graph shows the method allows the measurement of molecular weights of unknown proteins.

Besides calculations of molecular weight, capillary electrophoresis is capable of quantitative analysis based on the detection of ultraviolet radiation and the automation of measurement since no gel is used.

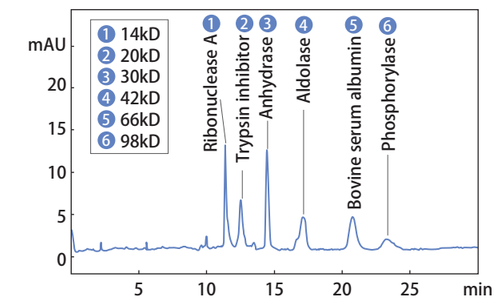


Fig. 1 Separating proteins of different molecular weights

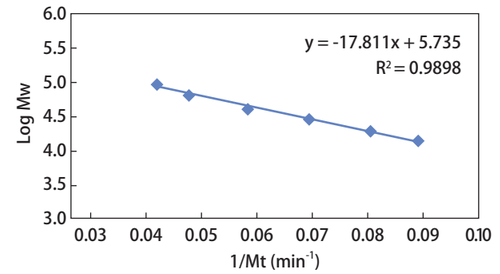


Fig. 2 Plots of reciprocal of detection times and Log Mw

11 CE Analysis of proteins by isoelectric focusing

Separation analysis

Purpose The need to analyze isoelectric point has grown across biotechnology, pharmaceuticals, and similar fields. Such analysis would allow the identification of substances of the same molecular weight and different isoelectric points as well as evaluations of physical properties like purity and identity for proteins and IgG antibodies.

Although polyacrylamide gel isoelectric focusing is widely known for isoelectric point measurements, the method is associated with various problems, including complex procedures related to gel preparation and long measurement times.

We performed isoelectric point analysis out using a capillary electrophoresis apparatus.

Result Figures 1 and 2 show the results of isoelectric focusing. Figure 1 shows the results of measurement using ampholyte (amphoteric carrier) pI 5-8. Figure 2 shows results using ampholyte pI 3-10. In each case, we see sharp peaks. The measurement can be done quickly, within 30 minutes. The isoelectric point measurements also cover a broad range from pI 3 to 10.

The results indicate isoelectric points can be quickly and easily measured using a capillary electrophoresis apparatus.

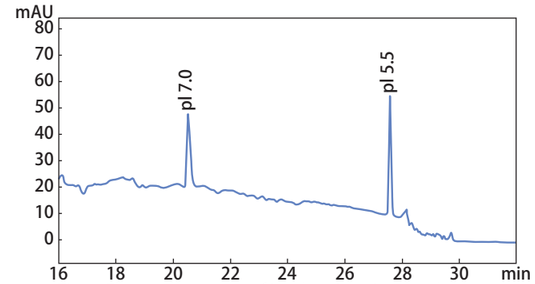


Fig. 1 Electropherogram of isoelectric point standard marker (pI 7.0, pI 5.5)
*We used Ampholyte 5-8.

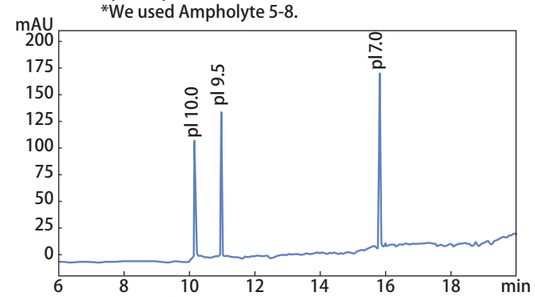


Fig. 2 Electropherogram of isoelectric point standard marker (pI 10.0, pI 9.5, pI 7.0)
*We used Ampholyte 3-10.

12 CE Analysis of DNA by the linear polymer sieving method

Separation analysis

Purpose A current need in the fields of pharmaceuticals, drug production, and biotechnology is a method allowing rapid and easy high-resolution measurement method of DNA fragments. To date, DNA analysis has been done by the molecular sieve method using a gel-filled capillary tube. However, this method poses various problems, including the complexity of the method required to prepare the gel, the need to prepare a gel-filled capillary for each analysis, and the difficulty of automating analysis.

We used a carrier solution containing linear polymer to eliminate the complexity of preparation and to achieve fast, easy, automated measurements.

Result Figures 1 and 2 show results for DNA fragments. The analyses are capable of separating fragments after the user supplies a carrier solution containing linear polymer.

The ability to perform analysis simply by filling with a carrier solution also allows continuous analysis. We required as little as 15 minutes for each analysis.

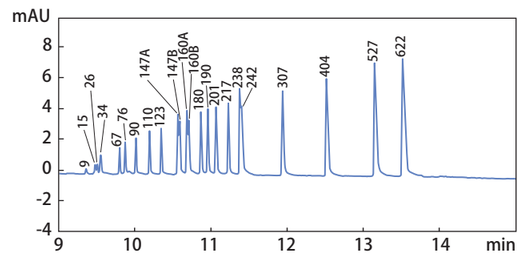


Fig. 1 Electropherogram of DNA standard marker (Marker 10: 0.5 μg/μL)

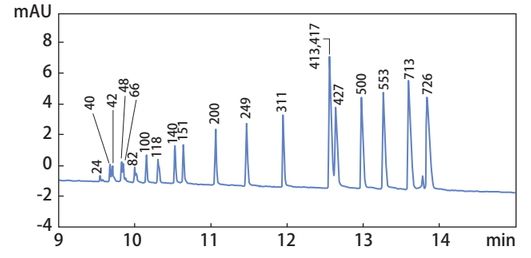


Fig. 2 Electropherogram of DNA standard marker (Marker 9: 0.5 μg/μL)

13 ELSZ Severe test evaluation of eye drops by particle diameter measurement

Particle diameter

Purpose Pharmaceuticals are tested to confirm that they meet the requirements of the stability test guidelines of the Ministry of Health, Labor and Welfare before approval for their introduction is granted. All companies perform various severe tests at the preliminary stage of drug development. For eye drops, a nonionic surfactant is added to form micelles that include a fat-soluble active ingredient. In nonionic surfactants, we observe a phase transition temperature called the cloud point. At temperatures above the point, the surfactant will cause the eye drop to become cloudy, potentially impairing its product value.

We used our ELSZ series zeta potential/particle diameter measurement system to measure average particle diameter and the scattering intensity of commercially available eye drops after raising temperatures.

Result Measurement was performed as temperatures rose from 20ºC to 80ºC at intervals 10 minutes for temperature control. The average particle diameter at 20ºC is about 9.8 nm, increasing with temperature to about 21 nm at 76ºC and to about 426 nm at 78ºC. The emergence of the clouding phenomenon appears to indicate a phase transition of the nonionic surfactant occurred at this temperature.

Based on the distribution of particle diameters (data is not displayed), the presence of a certain amount of aggregate is recognized from about 42ºC. Product quality may be affected from this temperature.

Based on the results, we assume that significant effects on quality begin to emerge around 78ºC, with lesser effects beginning to emerge at around 42ºC.

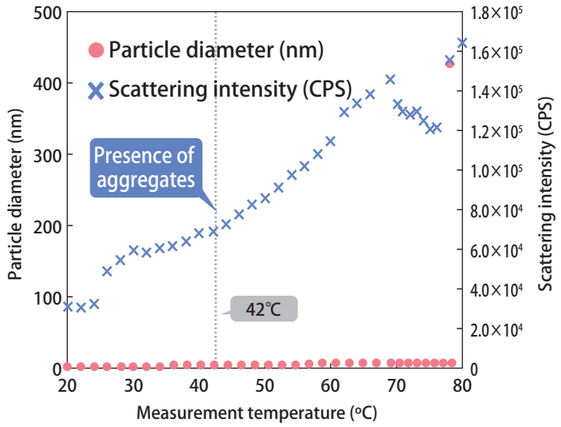


Fig. 1 Average particle diameter of micelles contained in eye drops and scattering intensity

14 CE Analysis of antibiotics

Separation analysis

Purpose Antibiotics like penicillin and streptomycin are compounds produced by microorganisms with human medical applications. They are also widely used in agricultural, animal husbandry, and fisheries applications.

We analyzed specimens of ampicillin, vancomycin, and cefazolin, certain amounts of which were prepared as dry preparations in each well on microplates. Separation was performed by micellar electrokinetic chromatography (MEKC).

Result Figure 1 shows examples of measurements of ampicillin. Figure 2 shows examples of measurements of vancomycin and cefazolin.

We find that three kinds of antibiotics can be analyzed. The broad peaks found besides each antibiotic are assumed to be peaks of other components present in the microplates.

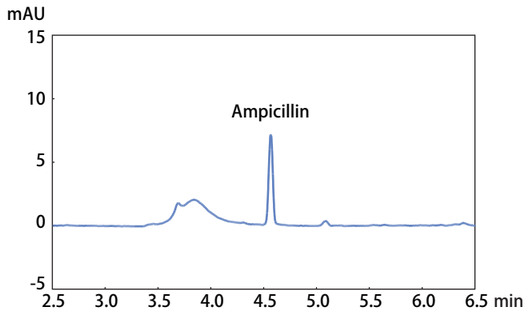


Fig. 1 Electropherogram for ampicillin

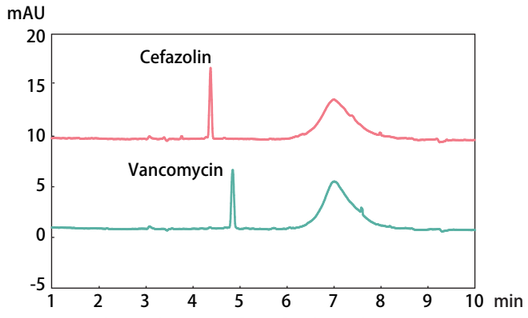


Fig. 2 Electropherogram for cefazolin and vancomycin

15 ELSZ Heat transition evaluation of IgG antibody by dynamic light scattering

Particle diameter Zeta potential

Purpose Antibodies are glycoprotein molecules that recognize and bond to antigens, including specific proteins. They play an important role in preventing infections.

Since aggregates of such antibodies affect their function, evaluations of the physical properties of antibodies is important.

We used bovine-derived IgG to measure particle diameter and zeta potential and studied the heat transition conditions.

Result Figure 1 shows the temperature dependence of the average particle diameter and zeta potential of IgG. The average particle diameter of IgG remained near-constant at around 15 nm from 20ºC to 62ºC.

The average particle diameter IgG increases in the range above 64ºC, and aggregates of several μm form at 68ºC. The plots indicate the heat transition temperature of IgG is 62ºC.

Zeta potential changes in a manner similar to the change in average particle diameters.

Figure 2 shows the distribution of particle diameters for IgG at each temperature, indicating a stepwise temperature-dependent association/aggregation for IgG.

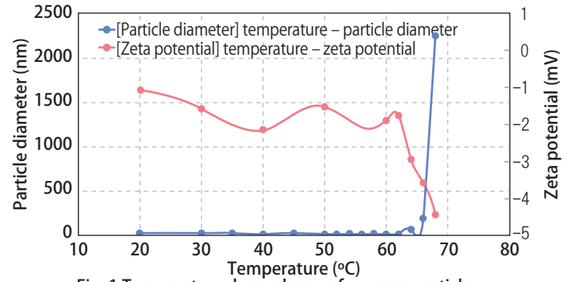


Fig. 1 Temperature dependence of average particle diameter and zeta potential of IgG

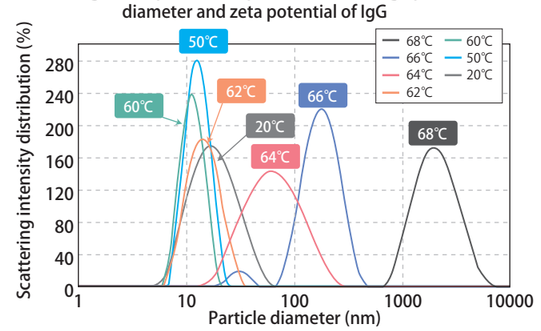


Fig. 2 Distribution of particle diameters for IgG

16 ELSZ Heat denaturation analysis of lysozyme by dynamic light scattering

Particle diameter

Purpose Lysozyme is an enzyme that hydrolyzes the polysaccharides that form the cell walls of eubacteria found in human tears, nasal discharge, and breast milk. Industrially, hen egg-white lysozyme extracted from egg-white is used in the food industry to improve shelf life of food products and in the pharmaceutical industry as a cold medicine ingredient. Heat denatures, causes conformational change, and increases the hydrophobicity of lysozyme, resulting in precipitation.

Understanding the specific structures involved in the heat denaturation process is important for supporting stability and steric structure formation.

We used dynamic light scattering to evaluate the heat denaturation process, including the effects of temperature on particle diameter and relative light scattering intensity.

Result Based on Figure 1, both cumulant average particle diameter and relative light scattering intensity rapidly increase above 70ºC, suggesting the onset of heat denaturation. Average particle diameter increases from the nano order to the micron order, while relative light scattering intensity rises 106-fold, on the basis of which we can assume aggregation and a clouded state.

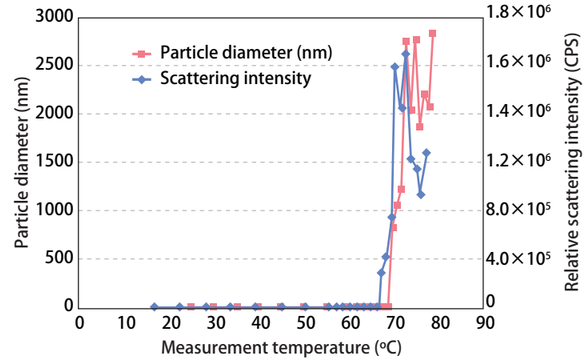


Fig. 1 Temperature dependence of lysozyme

17 CE Analysis of peptide-based hormones

Separation analysis

Purpose Hormones are chemical substances synthesized and secreted by animals that effect change through bodily fluids and incite action in certain specific tissues. Hormones can be peptides, steroids, and catecholamines, with peptides the most abundant. Here we introduce an example of the separation of four kinds of peptide hormones: bradykinin, angiotensin, somatostatin, and leucine-enkephalin.

Result Figure 1 shows an example of the measurement of a mixed solution of four kinds of peptide hormones. The charge of a peptide varies with pH and the sum of amino groups at N end, carboxyl groups at C end, and dissociative groups in side chains of acidic amino groups and basic amino groups. Since electrophoretic mobility is determined by charge and the size of the compound, depending on the composition of the amino acid, peptides exhibit unique pH dependencies on electrophoretic mobility. Figure 2 shows the results.

Using this pH dependence, capillary electrophoresis can separate most peptide hormones.

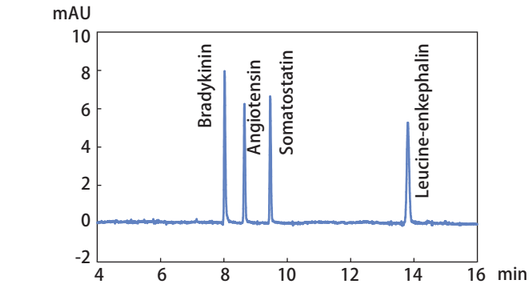


Fig. 1 Electropherogram of four kinds of peptide hormones (100 ppm each)

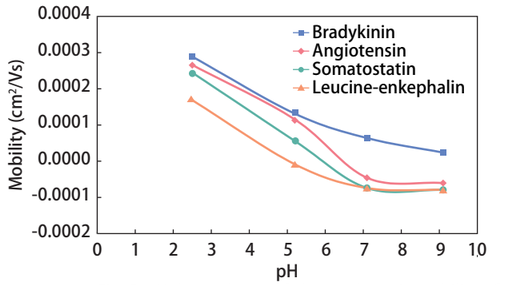


Fig. 2 pH dependence of electrophoretic mobility of four kinds of peptide hormones

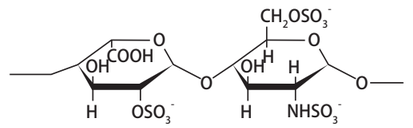
18 CE Analysis of heparin and an analogue

Separation analysis

Purpose As shown in Figure 1, heparin is a polyelectrolyte polysaccharide composed of sulfate groups and carboxyl groups and with a main structure of two repeated monosaccharides. It is an essential additive for dialysis. In 2008, the release of heparin preparations containing impurities led to hypotensive symptoms, and even deaths, in patients receiving injections of the preparation in the USA and Germany. The United States Food and Drug Administration (FDA) selected measurement by capillary electrophoresis or NMR as an effective method for inspecting heparin and released examples of measurement. In the measurements by capillary electrophoresis publicized by the FDA, a sharp subpeak preceded the peak of heparin for the heparin preparation with which the abnormality occurred.

We studied the sharp peak using capillary electrophoresis.

Result Heparin has three sulfate groups in each unit of two monosaccharides. Excessively sulfated chondroitin has four sulfate groups in each unit of two monosaccharides. Since the latter is not readily available, we used dextran sulfate, which has four to six sulfate groups in each unit of two monosaccharides. Figure 2 shows the electropherogram obtained. Dextran sulfate moves to the positive pole faster than heparin. In measurements involving a mixture, we identified a sharp peak appearing before the main peak of heparin. This is very similar to what was presented by the FDA for the heparin preparation in question and indicates capillary electrophoresis is useful in detecting components with different sulfate group contents in units of two monosaccharides.



・L-iduronic acid 2-sulfate residue ・N-sulfo-D-glucosamine 6-sulfate residue

Fig. 1 Structural formula of heparin

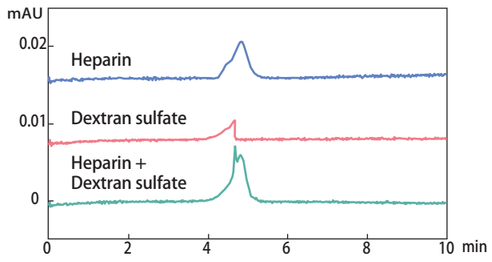


Fig. 2 Electropherogram of heparin and an analogue

19 CE Analysis of IgG antigen by molecular sieve in the presence of SDS

Separation analysis

Purpose The mainstream for protein electrophoresis includes SDS-polyacrylamide gel electrophoresis and acrylamide gel isoelectric focusing. Capillary electrophoresis is also capable of gel electrophoresis, but poses problems related to the complexity of filling gel and reproducibility. In recent years, as solutions of linear polymers were found to have molecular sieve effects (Linear Polymer Sieving: LPS), capillary electrophoresis entered use for ease of use.

We performed electrophoresis measurement of bovine-derived IgG antigen by LPS separation mode in the presence of SDS.

Result Figure 1 shows the result of measurements of reduced and unreduced IgG antigens. The reduced IgG antigen shows two peaks: one for the L chain and one for the H chain.

Since no other peaks are detected, an IgG with a different sugar chain is assumed to be absent. On the other hand, since most of the peaks are associated with an unreduced IgG antigen, fragmented IgG appear to have been detected.

Thus, capillary electrophoresis is an effective means for characterizing antigens.

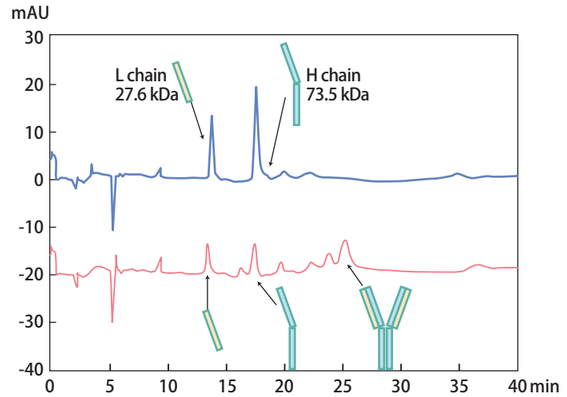


Fig. 1 Electropherogram of reduced and unreduced (bovine-derived) IgG antigens

20 ELSZ Particle diameter evaluation of different kinds of liposomes

Particle diameter

Purpose Liposomes are generally classified as small unilamellar liposomes (small unilamellar vesicle, SUV; 100 nm or less), large unilamellar liposomes (large unilamellar vesicle, LUV; 100 to 500 nm), and multilamellar liposomes (multilamellar vesicle, MLV; 200 to 1000 nm).

We performed particle diameter measurement of these three kinds of liposomes, each prepared by ultrasonic treatment, the reverse phase evaporation method, and the vortex processing method, by dynamic light scattering. Each sample was prepared to a concentration of 5 mM, with a focus on the phosphate part of the phospholipid. To prevent the collapse of the liposome, we removed dust with a polycarbonate filtration membrane with 0.4 μm circular holes.

Result The figure on the right shows the results of evaluations of the distribution of particle diameters obtained with each sample of SUV, LUV, and MLV. We obtained different particle diameters depending on the preparation method.
Dynamic light scattering allows evaluations of the distribution of particle diameters of liposome in about 3 minutes per sample. If the obtained distribution is not as desired, you can review the material, conditions, and preparation method and remake the samples. Particle size evaluation is an important index for this purpose.

Sample source: Visiting professor Tetsuro Yoshimura, Liposome Bioengineering Laboratory, Faculty of Engineering, Graduate School of Engineering, Mie University

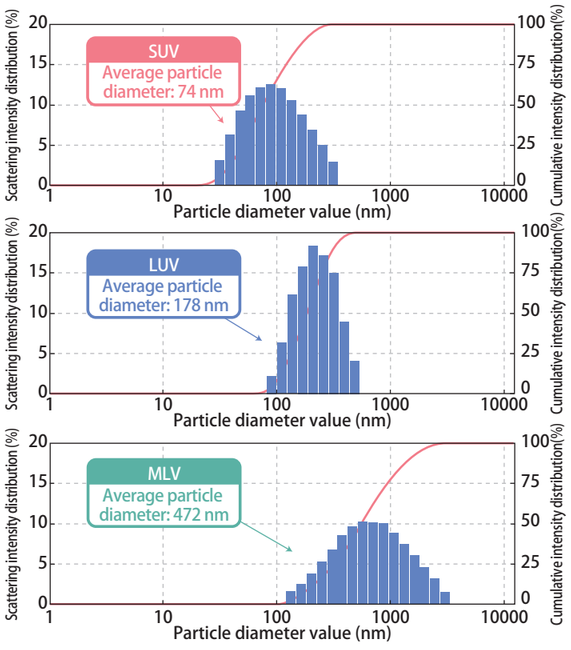


Fig. 1 Distribution of particle diameters of various liposomes

21 ELSZ Evaluation of particle diameter and zeta potential of DDS nano-material liposomes

Particle diameter | Zeta potential

Purpose Closed vesicles consisting of lipid bilayers, liposomes have drawn attention as potential carriers of drugs for chemotherapy. They offer useful properties as drug carriers because their lipid composition, particle diameter, and surface charge are readily adjusted. Their surfaces can be modified with anti-tumor peptides and antigens, making it easy to target specific substances. Thus, evaluations of physical properties (measurements of particle diameter, surface potential, and so on) are critical to understanding the pharmacokinetics of liposomes.

Result We measured the particle diameter and zeta potential of liposomes (Liposome KIT L-α-Phosphatidylcholine [egg yolk] for positively charged liposomes from Sigma). The sample was dispersed in saline. After passage through a filter with pore size of 0.2 µm, we measured particle diameters by dynamic light scattering and measured zeta potential by the Laser-Doppler method. Figure 1 shows the average particle diameter and the distribution of particle diameters. Figure 2 shows zeta potential.

Zeta potential was found to be a positive charge, as indicated in the catalog.

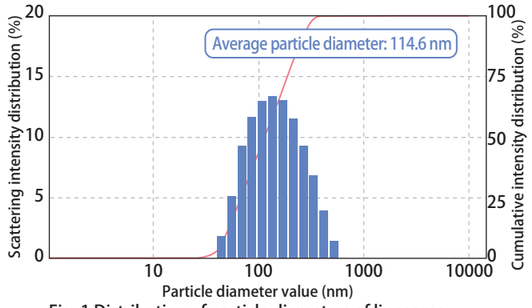


Fig. 1 Distribution of particle diameters of liposome

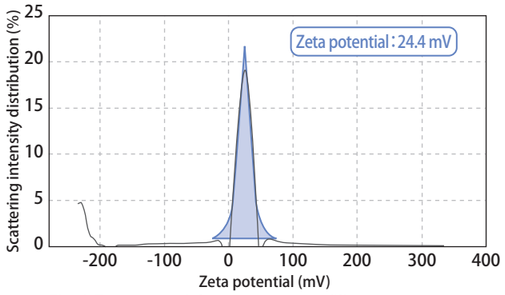


Fig. 2 Zeta potential measurement of liposome

22 ELSZ Isoelectric point evaluation of DDS nano-material liposome

Particle diameter | Zeta potential

Purpose The point at which the zeta potential becomes zero (surface potential of the particle becomes zero) is called the isoelectric point. Colloid particles lose their electrostatic repulsion to aggregate if the pH approaches the isoelectric point. To stabilize dispersion states, we must keep pH far from the isoelectric point to raise the absolute value of the zeta potential.

We measured the pH dependence of particle diameter and the zeta potential of liposomes (Liposome KIT L-α-Phosphatidylcholine [egg yolk] for positively charged liposome from Sigma).

Result We dispersed the liposomes in saline. After passing the solution through a filter with a pore size of 0.8 µm, we measured particle diameter and zeta potential for each pH value. Figure 1 shows the results. The isoelectric point was determined to be around pH 11.5. The average particle diameter at this pH is about 1 µm, larger than the value measured at other pH values. Since the absolute value of the zeta potential is larger in the neutral to acid region and the average particle diameter is also small, dispersion stability is good, due to electrostatic effects.

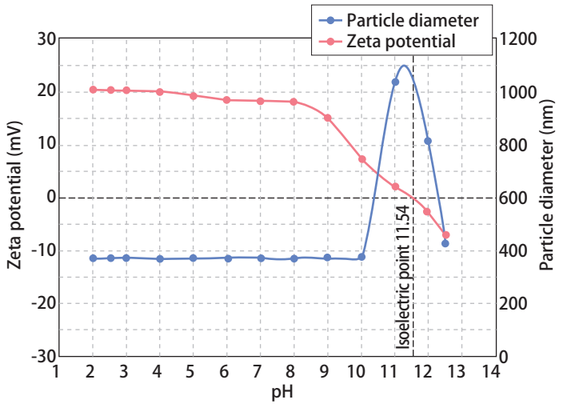


Fig. 1 pH titration measurement of liposomes dispersed in saline

23 ELSZ Evaluation of fixed aqueous layer thickness of liposomes

Zeta potential

Purpose Liposomes, which have drawn attention as drug carriers for chemotherapy and other therapies, have a bilayer, much like cell membranes in vivo. Specific properties can be conferred on liposomes by enclosing drugs in the bilayer or by adding charged substances and polymers to the surface. In recent years, measurements of the fixed aqueous layer thickness (FALT) of liposomes has been often performed to evaluate drug release and drug effects. The thickness can be derived from the equation representing surface potential, as shown below.

$$\Psi(x) = \Psi_0 \exp(-\kappa\Psi x) \quad \cdots (1)$$

Here, $\psi 0$: interfacial potential, κ: Debye-Hückel parameter ($\kappa \fallingdotseq 3.3\, z \sqrt{C}$, C is electrolyte concentration, z is valence), x: distance from particle surface
Since the zeta potential (ζ) is defined as the potential at sliding surface (L),

$$\zeta = \Psi(L) \quad \cdots (2)$$

From equations (1) and (2), $\Psi(L) = \Psi_0 \exp(-\kappa L)$ ..... (3)

Taking the log of both sides, we get, $\ln(\Psi(L)) = \ln \Psi_0 - \kappa L$ ..... (4)

When zeta potentials are determined at different salt concentrations and ln (ψ (L)) plotted against κ, we can determine the position of L (the sliding surface) from the slope. This position is defined as the fixed aqueous layer thickness.

We used liposomes (Liposome KIT L-α-Phosphatidylcholine [egg yolk] for positively charged liposome from Sigma) dispersed in 0.005 to 0.2 M/L aqueous NaCl solution and measured the zeta potential. The liposome concentration was 0.5 to 1 mg/ml.

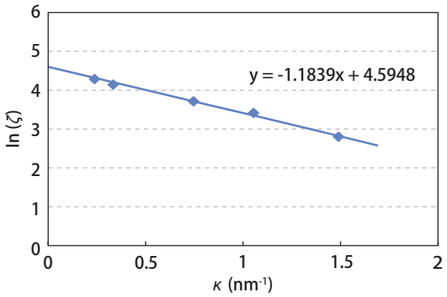


Fig. 1 Plot (ln (ζ) − κ) for calculation of fixed aqueous layer

Result Figure 1 shows the plot of ln (ζ) against κ. Based on its slope, the aqueous layer thickness is calculated to be 1.2 nm.
To date, zeta potential has been evaluated as a surface potential of a particle. Since the zeta potential can be further evaluated as the fixed aqueous layer thickness of particle, its utility is expected to rise in the field of DDS.

24 ELSZ Zeta potential evaluations of a complex of cells cultured on substrate and their polyions

Zeta potential

Purpose In the electrostatic interaction of biomaterials with living tissues, the surface potential on flat materials (cells and skin surfaces) have been rarely evaluated. We prepared human umbilical vein endothelial cells (HUVEC) cultured on polystyrene substrates and examined the effects of different polyion complexes (PIC) adsorbed to their surfaces and electrostatic interactions. PICs were prepared at different mixing ratio of anionic and cationic polymers (cation/anion, C/A ratio). The HUVEC cells were cultured using Endothelial Cell Basal Medium as the culture medium for three days on 1.4 cm × 3.4 cm polystyrene substrate at 37°C, 5% CO2 and 1.2 × 10^4 cells/cm^2, and made confluent. PICs were prepared to achieve C/A ratios of 1.5, 4, and 16 using poly (dimethylaminoethyl methacrylate) (PDMAEMA) as the cationic polymer and plasmid DNA as the anionic polymer. We performed measurements by injecting PBS solution in dedicated cells for flat surface potential measurements and setting the samples immersed in the PBS solution.

Result The surface potential of the polystyrene substrate for culturing cells was determined to be −18 mV. The surface potential of the substrate on which HUVEC cells were cultured was determined to be −8 mV. Figure 1 shows the results when the PIC solution is adsorbed to each substrates for three hours. For a C/A ratio of 0.5, the value obtained was almost identical to the surface potential of the substrate on which HUVEC cells were cultured. Along with increased C/A ratio, we observed a trend toward declining absolute values for surface potential due to the neutralization of cell surfaces.

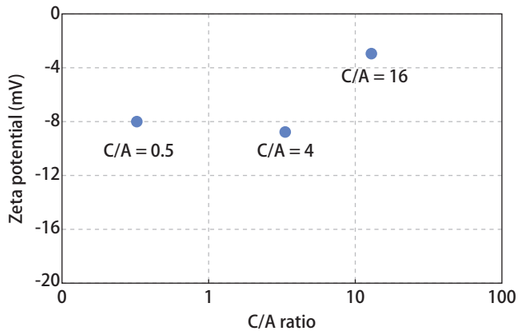


Fig. 1 Surface zeta potential when C/A ratio of PIC is varied

Sample source: Dr. Ryosuke Iwai, National Cerebral and Cardiovascular Center