

Introduction to Differential Sedimentation

Differential Centrifugal Sedimentation, or DCS (sometimes also called "two-layer" sedimentation) is a widely used analysis method that produces extremely high resolution size distributions of microscopic to sub-microscopic particles. The normal measurement range for the method is from about 0.01 micron (10 nanometers) to about 50 microns, though it is possible with some types of materials to extend the range to below 0.003 micron (3 nanometers) or up to 120 microns or more. This document provides some background information on particle size analysis by sedimentation, explains how the DCS method works and describes the advantages and limitations of the method. Several example analyses are presented to help illustrate the capabilities of DCS.

Basic Theory of Particle Size Analysis by Sedimentation

Sedimentation of particles in a fluid has long been used to characterise particle size distribution. Stokes' law¹ is used to determine an unknown distribution of spherical particle sizes by measuring the time required for the particles to settle a known distance in a fluid of known viscosity and density. Sedimentation can be either gravitational (1 g-force), or centrifugal (many g-force).

Gravitational sedimentation is normally limited to particles of relatively large size, because the rate of sedimentation for small particles is too low to give a practical analysis time, and because Brownian motion of small particles becomes too large to allow effective settling. A very narrow distribution of small particles will be reported as a broad distribution when the rate of particle diffusion is comparable to the sedimentation rate. Very small particles (<0.1 micron) never settle by gravity unless they are extremely dense, so most types of very small particles can not be measured by gravitational sedimentation. Sedimentation in a centrifuge extends the range of sedimentation analysis to much smaller particles. High g-force makes sedimentation of small particles much faster than Brownian diffusion, even for very small particles. When a centrifuge is used, Stokes' law must be modified to account for the variation in g-force with distance from the center of rotation.

$$D = \{ (18 \eta \ln (R_f / R_0)) / ((\rho_p - \rho_f) \omega^2 t) \}^{0.5} \quad (\text{Eq. 1})$$

Where: D is the particle diameter (cm)
 η is the fluid viscosity (poise)
 R_f is the final radius of rotation (cm)
 R_0 is the initial radius of rotation (cm)
 ρ_p is particle density (g/ml)
 ρ_f is the fluid density (g/ml)
 ω is the rotational velocity (radians/sec)
 t is the time required to sediment from R_0 to R_f (sec)

For a centrifuge running at constant speed and temperature, all of the parameters except time are constant during an analysis. The values for these are either well known or can be accurately measured. Within a broad range of analysis conditions, the modified form of Stokes' law accurately measures the diameter of spherical particles based on arrival time at the detector.

Methods of Sedimentation Analysis

There are two common sedimentation methods: integral, and differential. The following discussion explains the differences between these methods.

Integral Sedimentation

The integral method (Figure 1) is the oldest of the sedimentation methods. A detector beam (a light beam or X-ray beam) passes through the fluid at a known distance from the fluid surface, and measures particle concentration. The initial intensity of light or X-rays reaching the detector is a minimum, corresponding to the maximum concentration of particles. As particles settle through the fluid, the concentration of particles remaining in the dispersion falls, and the intensity of light or X-rays that reaches the detector increases. Stokes' law is used to calculate the size of particles that sediment out of the fluid as a function of time, and a particle size distribution is generated by plotting the measured concentration of particles against the calculated particle diameter. The result of the analysis is an integral representation of the particle size distribution. The method is called integral sedimentation because the sum (the "integral") of all particles smaller than a particular size is being continuously measured during the analysis. A differential particle size distribution can be generated from the integral results by applying mathematical differentiation with respect to diameter.

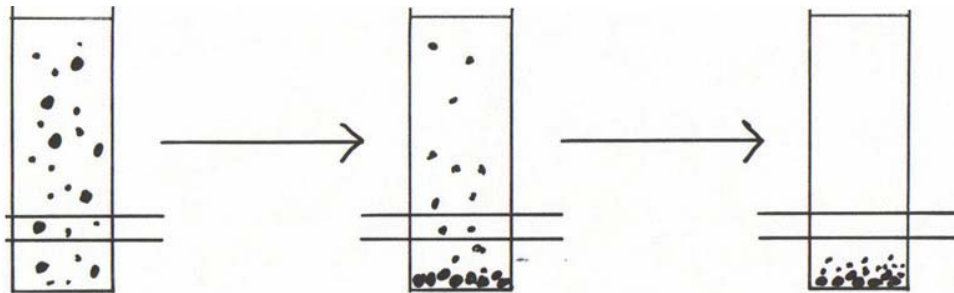


Figure 1 – Integral Sedimentation Method

Integral sedimentation can also be applied to particles lower in density than the fluid in which they are suspended. In this case, the particles have a net buoyancy, so they sediment toward the surface of the fluid rather toward the bottom.

There are three significant operational problems with integral sedimentation in a centrifuge. First, the initial conditions of the analysis are difficult to characterise. If the sample is added to a centrifuge that is already spinning, then there will be turbulent mixing of the sample dispersion as it is added to the centrifuge, which makes accurate measurement of sedimentation time difficult. If a sample is added to a centrifuge that is not spinning, and is later accelerated to high speed, then it is necessary to accurately measure and account for the changing speed during the acceleration period. It is also necessary to use a centrifuge of a design that ensures there is no mixing of the sample during acceleration. Second, convection currents can develop during an analysis unless the temperature of the sample is held constant; any convection currents in the fluid can reduce both resolution and the accuracy of results. High speed centrifuges generate frictional heat, which makes it more difficult to maintain constant temperature in the sample fluid. Third, the sedimentation chamber must be emptied and cleaned following each sample, which increases operator labour.

Differential Sedimentation

Differential sedimentation (see Figure 2) was first reported in 1930². A sample of particles to be analysed is placed on top of a column of clear liquid at the start of the analysis, and particles settle according to Stokes' Law, just as in integral sedimentation. The detector initially reads maximum intensity, but the signal is reduced when particles reach the detector beam. The reduction in intensity indicates the concentration of particles in the detector beam. When an X-ray beam is used, the reduction in intensity is proportional to particle concentration. When a monochromatic light source is used, Mie theory light scattering can be applied to the intensity data to calculate particle concentration.

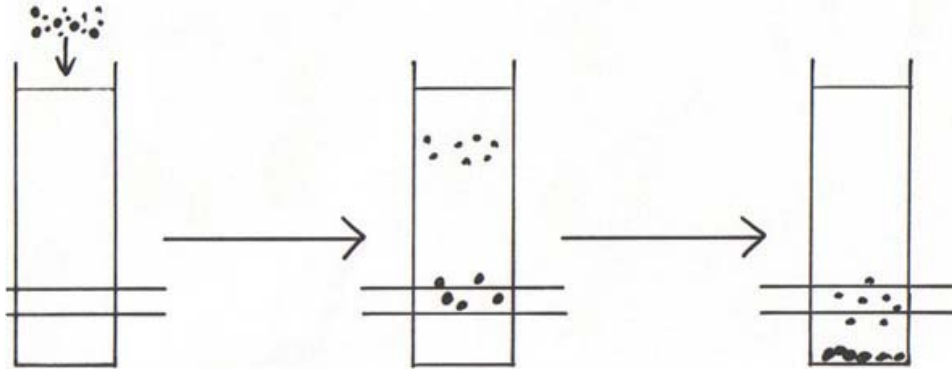


Figure 2 – Differential Sedimentation Method

When all particles have passed the detector, the signal returns to the original level. A plot of the particle concentration against the calculated particle diameter produces a differential distribution. At any time during the analysis, only particles of one particular size range are being measured by the detector beam; all larger particles have already passed the beam, and all smaller particles have not yet arrived. The method is called differential sedimentation because only a tiny part of the distribution (a "differential") is being measured by the detector beam at any time. An integral distribution can be generated from a differential distribution by applying mathematical integration with respect to particle diameter. A differential size distribution and its corresponding integral distribution are shown in Figure 3.

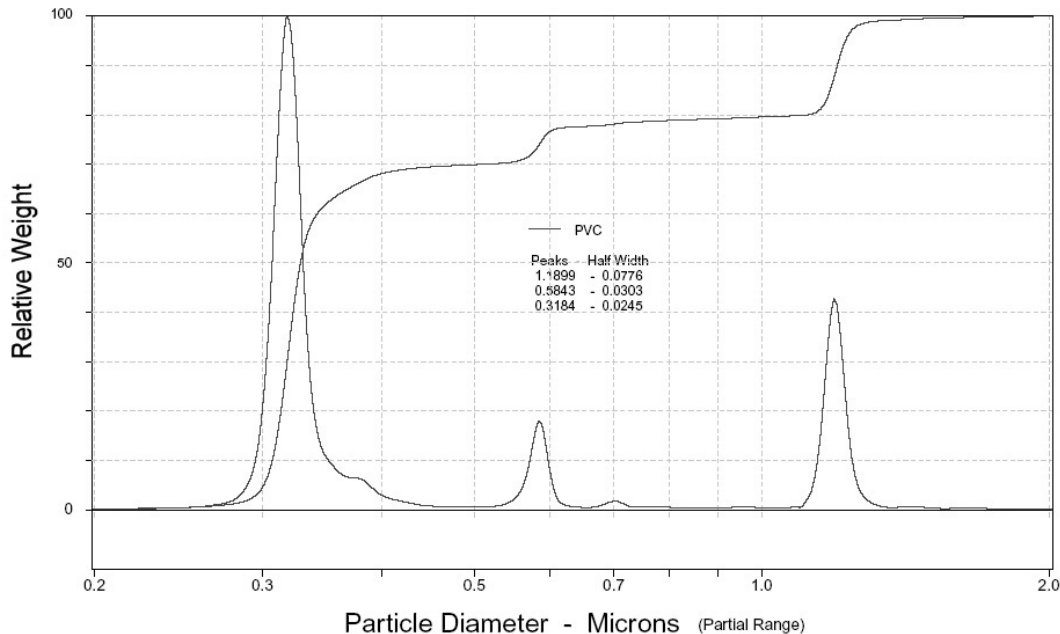


Figure 3 – Differential and Integral Distributions

Actually running a differential sedimentation is a little more complicated than suggested by the above description. When a sample of dispersed particles which are more dense than the fluid in the column is placed on top of the column, the particles do not settle individually according to Stokes' Law. Instead, the entire sample suspension rapidly settles as a bulk fluid through the liquid column, in exactly the same way as a homogeneous liquid of higher density (like 10% sodium chloride in water) would settle through a column of another liquid of lower density (like water). The bulk settling of a sample in differential sedimentation is commonly called "streaming" or "sedimentation instability"³. All information about the particle size distribution can be lost when streaming takes place. Several methods^{4,5,6} have been developed to eliminate streaming. Each of these methods is effective because a slight density gradient is formed within the fluid column, prior to starting analyses. A wide range of fluids can be used to form a density gradient. In aqueous systems, gradually changing concentrations of methanol, ethanol, glycerine, sucrose, and many other materials have been used. In nonaqueous systems, many mixtures of fluids of different density can be used.

A density gradient eliminates streaming because at all times during the analysis the net density of the fluid, which is the average density of fluid plus any suspended particles, increases continuously from top to bottom in the fluid column. The condition which guarantees stable sedimentation is given by Equation 2.

$$\delta\rho_{net} / \delta R \geq 0 \quad (\text{Eq. 2})$$

Where: ρ_{net} is the net fluid density (including liquid plus any suspended particles)
R is the distance from the center of rotation

When a small volume of a particle suspension is placed on the surface of the fluid column, the net density of the suspension is very slightly higher than the pure fluid; but the fluid just under the surface is also slightly higher in density than the pure fluid, due to the density gradient. There is no driving force for bulk settling of the particle suspension, so there is no instability, and the particles sediment through the fluid according to Stokes' Law. The required steepness of the density gradient depends upon the net density of the sample to be measured. A sample with higher net density (higher particle concentration and/or higher particle density) requires a steeper density gradient than a sample with lower net density. Most samples are diluted to low concentration, so only a very slight density gradient is required to insure stability. Density gradients of less than 0.01 g/ml per centimeter of fluid height are normally sufficient to insure complete stability.

A density gradient also eliminates thermal convection, so sedimentation is not disrupted by slight changes in fluid temperature during an analysis. Relatively large temperature changes (>0.5°C) can cause some loss of accuracy unless they are accounted for, because fluid viscosity changes with temperature.

Differential Centrifugal Sedimentation

DCS Instrument Design

The most common design for DCS instruments is a hollow, optically clear disc that is driven by a variable speed motor. A typical disc cross section is shown in Figure 4. The disc can be of virtually any size, but manufacturers have settled on a diameter of approximately 125 to 150 mm. The detector beam is usually monochromatic light of relatively short wavelength (400 nm - 500 nm); though some instruments use a longer wavelength (~650 nm), or X-rays. Shorter wavelength light gives better detector sensitivity when particles smaller than 100 nm are measured.

To prepare the instrument for analysis, the disc is set in motion at constant speed, and then the disc chamber is filled with a fluid which contains a slight density gradient. Samples are prepared for analysis by dilution in a fluid of slightly lower density than the least dense fluid in the disc. The lower density fluid used for the sample reduces initial mixing of the fluid inside the disc with the sample. When a sample is injected (normally using a small syringe), it strikes the back inside face of the disc, and forms a thin film, which spreads as it accelerates radially toward the surface of

the fluid. When the sample dispersion reaches the fluid surface, it quickly spreads over the surface, because it is of lower density (it "floats" on the higher density fluid). Once a sample is on the fluid surface, sedimentation of individual particles begins. The injection of a sample is rapid (typically <50 milliseconds), so the starting time for an analysis is well defined, and the precision of sedimentation time is quite good.

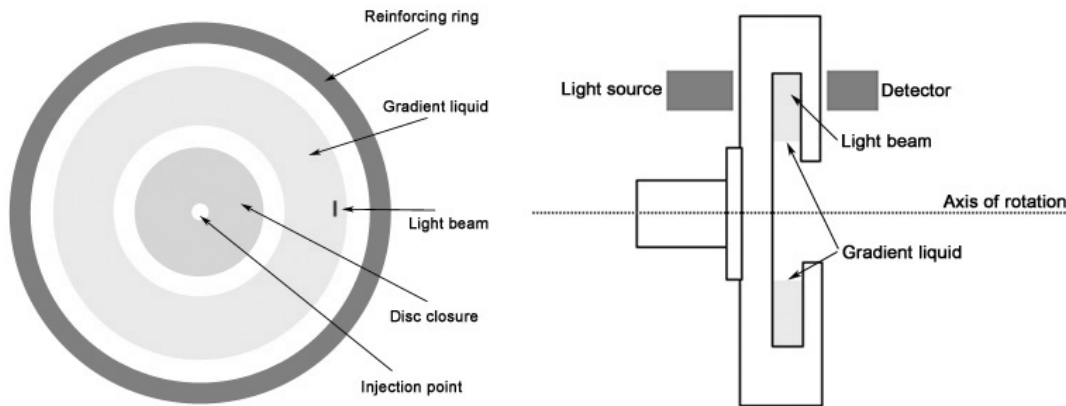


Figure 4 – Hollow Disc Centrifuge Design

When an analysis is complete, the instrument is ready for the next sample. There is no need to empty and clean the centrifuge, so many samples can be run in sequence without stopping the centrifuge. The only limitation on continuous run time is that the density gradient slowly degrades due to molecular diffusion. When the density gradient is no longer steep enough to maintain stable sedimentation, the instrument must be stopped, emptied, and a new gradient formed. Typical gradient lifetime is 2 to 72 hours, depending on the molecular weight and viscosities of the materials that form the gradient.

Advantages and Limitations of the DCS Method

All methods of particle size analysis can be characterised by three parameters:

- the **accuracy** of the reported size distribution;
- the **repeatability** of the reported size distribution;
- and the **resolution** of the distribution.

Accuracy and Repeatability

Accuracy and repeatability of the DCS method are very good in nearly all cases. Any significant inaccuracy in the results is caused by either inaccurate values for the physical parameters of the system (densities, viscosity, rotational speed, etc.), instability in the sedimentation, or by deviation of the sedimentation from Stokes' Law.

- Physical Parameters

The overall accuracy of the analysis depends upon the combined accuracy of each of the values in Equation 1. For example, if the viscosity of the fluid is actually 2% higher than entered in Equation 1, then the reported particle size will be about 1% smaller than correct. It is possible to achieve nearly any desired level of accuracy by improving the accuracy of the parameters in Equation 1. An alternative method to improve accuracy is to use a narrow calibration standard of precisely known size to determine the effective combined value, K, for all the parameters in Equation 1. Equation 1 then reduces to:

$$D = K (1/t)^{0.5} \tag{Eq. 3}$$

Where: K is a combination of constants
 t is time to reach the detector



A calibration standard can be used externally, where it is analysed just before or just after an unknown sample to determine K, or internally, where a small amount of the calibration standard is added to the unknown. Instrument software finds the calibration peak within the distribution of the unknown sample, and adjusts the value for K so that the calibration standard peak is exactly the correct diameter. The adjusted value for K is applied to the entire distribution, so the accuracy of the analysis improves. Internal calibration gives extremely high accuracy and repeatability: the peak sizes in replicate analyses of an unknown are usually within +/- 0.25% when an internal standard is used.

- Sedimentation Stability

Any instability (streaming) during an analysis reduces both accuracy and resolution. Streaming causes the reported size distribution to be larger than correct, because during streaming particles move toward the detector faster than they would in normal sedimentation. Streaming usually takes place near the beginning of an analysis, when the entire sample is contained in a thin fluid layer near the surface. A small amount of streaming will cause the sample to form a broad initial band, followed by normal sedimentation; the result is both lower resolution and larger than correct reported sizes.

Commercial DCS instruments are normally set up to operate under conditions that always yield stable sedimentation. However, to verify that the sedimentation is stable, a direct means of confirming stability is needed. Some DCS instruments are equipped with a strobe light which is synchronised with the rotation of the centrifuge. This allows direct visual observation of the stability of sedimentation. With experience, an operator can judge if there is any instability based upon the appearance of the sedimentation. Other instruments rely on a narrow calibration standard to verify stability. When a calibration standard is used (either internal or external), evaluation of sedimentation stability can be made automatic; the instrument software can compare the measured width and shape of the calibration standard peak with the known width and shape for that calibration standard. Any significant change in distribution width or shape indicates instability in the sedimentation.

- Deviation from Stokes' Law

Stokes' law does not accurately describe the sedimentation process if the Reynolds number for the system becomes too high. The Reynolds number increases with larger particles, faster sedimentation rate, and lower fluid viscosity. Most sedimentation analyses are run at low Reynolds numbers (<0.02), where deviation from Stokes' law is less than 0.5%. For example, at a centrifuge speed of 10,000 RPM, analysis of acrylic latex particles of 3 microns (density 1.13 g/ml) in water, will produce a Reynolds number of ~0.007, and a deviation from Stokes' law of ~0.25%. In cases where the Reynolds number is higher, deviation from Stokes' law can be taken into account by the instrument software so that the reported particle size distribution is accurate, regardless of Reynolds number.

Resolution

Compared to most other particle size analysis methods, DCS gives distributions that have excellent resolution. Calibration standards with very narrow distributions can be routinely resolved when the ratio of diameters is ~1.05, and partially separated when the ratio is as little as ~1.02.

What is Resolution?

Resolution of a size measurement method is the ability of the method to see a size distribution clearly. All size measurement methods report a distribution that is more or less "fuzzy" compared to the true distribution, much as an out of focus lens produces a fuzzy image. A lens can be very slightly out of focus (higher resolution) or far out of focus (lower resolution). Different particle sizing methods and different instruments have vastly different resolutions, even though nearly all particle sizing instrument manufacturers claim that their instruments have "high resolution". In order to rationally evaluate instrument resolution, we must first have a clear definition of resolution. For this document, resolution of a size measurement method is defined in two ways, which give the same result. First, resolution is the minimum fractional size difference between two

perfectly narrow families of particles which allows the two reported peaks to overlap by less than 5% of their total area. Resolution is stated as a percentage:

$$Resolution = 200 * (D_1 - D_2) / (D_1 + D_2) \tag{Eq. 4}$$

Where: D_1 is the diameter of the larger family
 D_2 is the diameter of the smaller family

For example, if we find that two families can be resolved with D_1 of 1.05 micron and D_2 of 0.95 micron, then the instrument resolution is 10%. Second, we can express the same resolution value in terms of the reported peak width for a single family of particles that are perfectly uniform in size, compared to their reported median diameter (D_{50}):

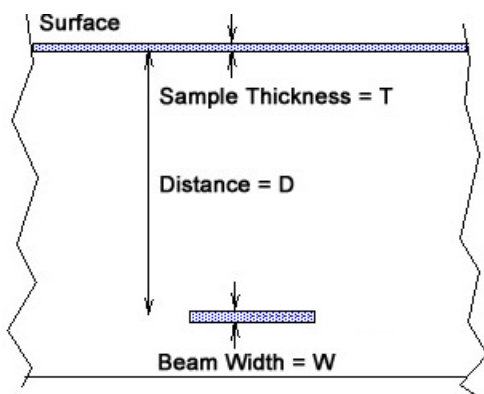
$$Resolution = 100 * (D_{95} - D_5) / D_{50} \tag{Eq. 5}$$

Where: D_{95} is the diameter larger than 95% of the entire reported distribution
 D_5 is the diameter larger than 5% of the entire reported distribution

These two resolution calculations give the same value for resolution.

Theoretical Resolution of the DCS

Particles sediment in the DCS according to Stokes' Law. Particles sediment at rates that are proportional to the square of the particle diameter; 1 micron particles sediment 4 times faster than 0.5 micron particles. At the start of a typical analysis, all particles are located in a thin band at the fluid surface. When particles arrive at the instrument's detector beam, they have separated from particles of different size, so the detector beam measures only a small slice (a "differential") of the whole size distribution. This is why we call the disc centrifuge method "differential sedimentation". Figure 5 shows a close-up of the sedimentation process.



The theoretical resolution depends on three factors:

- the width of the detector beam;
- the thickness of the initial sample band;
- the sedimentation depth.

As the sedimentation depth increases, the theoretical resolution increases as well, because the physical separation of particles of different size becomes larger. The following equation calculates the resolution as a function of the three variables shown in Figure 5.

Figure 5 – Resolution of the DCS

$$R = 100 * (((1 + (2T + W) / D)^{0.5}) - 1) \tag{Eq. 6}$$

The detector beam width is approximately 0.5 mm. The sedimentation depth depends on how much fluid is added to the centrifuge, but with a typical set-up of the instrument, the depth is in the range of 10 mm. The initial sample band width depends on the volume of sample that is injected into the disc. With a sample volume of 0.1 ml, the initial sample ring has a thickness of approximately 0.066 mm. Using these typical values, we can calculate the theoretical resolution: ~3.11%.

The theoretical resolution can be improved by reducing the detector beam width, increasing the sedimentation depth, and reducing the thickness of the initial sample band. For example, if the sedimentation depth is increased to 20 mm and the sample volume is reduced to 0.05 ml, then the theoretical resolution improves to ~1.4%. This means that two perfectly narrow peaks only 1.4% different in diameter could be completely resolved.

Factors that Reduce Resolution of the DCS

Actual instrument resolution is always slightly worse than the theoretical resolution described above. There are three factors that all can reduce resolution. These factors are: Brownian motion of the particles during sedimentation, sedimentation instability (streaming), and a broader than expected initial sample band that comes from the injection process. Each of these potential broadening factors is discussed below.

- Brownian Motion

Random diffusion of particles during the sedimentation will cause some particles to arrive at the detector beam earlier than expected (larger apparent diameter), and some particles to arrive later than expected (smaller apparent diameter). Brownian motion is a true diffusion process, with a calculable diffusion constant that depends on both particle size and fluid viscosity. In general, the mean absolute diffusion distance during a brief time (say 1 second) is proportional to the inverse square root of the particle diameter. A “random-walk” simulation of Brownian motion shows how the diffusion progresses.

If we were to measure a perfectly narrow family of 0.3 micron particles that required 12 minutes to reach the detector beam, then the band would reach the detector with an increase in band width equal to ~0.125 mm. In order to estimate the effect of Brownian motion on resolution, we can add the Brownian diffusion to the initial sample thickness. This yields an estimated resolution of:

$$100 * (((1 + (2T + W) / D)^{0.5}) - 1) =$$

$$100 * (((1 + (2(0.033+0.125) + 0.5) / 20)^{0.5}) - 1) = 2.02\%$$

After accounting for the effects of Brownian motion over 12 minutes, the DCS should still resolve peaks near 0.3 micron diameter that differ by as little as 2%, compared to 1.4% in the absence of Brownian motion.

For particles larger than 0.3 micron, or particles with sedimentation times less than 12 minutes, the effect of Brownian motion on resolution will be considerably less. For example, 0.5 micron particles arriving at the detector after 4 minutes form a band less than 0.02 mm wider than the initial sample thickness, so the resolution in this case would be ~1.5%, only very slightly different than the resolution would be without Brownian motion.

For particles that are significantly smaller than 0.3 micron, or that reach the detector more slowly, the effect of Brownian motion will be considerably more. For example, 0.05 micron particles that require 45 minutes to reach the detector will arrive as a band ~0.4 mm wide; with a total sedimentation depth of 20 mm, resolution in this case would be ~3.8%. Particles of 0.05 micron diameter that reach the detector after 90 minutes form a band that is about 0.75 mm wide; with a total sedimentation depth of 20 mm, the resolution in this case would be ~7.9%.

- Wall Effects

Particles that are near a wall of the centrifuge chamber sediment more slowly than particles that are far from either wall. There appear to be two different reasons for this slower sedimentation:

- Deviation from Stokes' Law due to the wall.
- Attraction/adhesion of particles to the wall, plus microscopic imperfections that can temporarily hold particles.

The combined effects are difficult to accurately quantify, but are significant.

- Initial Sedimentation Instability

All analyses in the DCS must be conducted in the presence of a density gradient, where the fluid at the outside edge of the disc chamber is of slightly higher density than the fluid near the surface. In the absence of a density gradient, differential sedimentation is unstable: an injected sample sediments “en-masse” rather than as individual particles. This instability is sometimes called “streaming”. The instability is caused by the effect of the (more dense) suspended particles on the net density of the fluid in which they are suspended. If the net density of the sample suspension is higher than the fluid inside the rotating disc, then the sedimentation will become unstable. During the entire analysis, the fluid that is just “below” a band of particles (that is, fluid slightly further from the center of rotation) must be equal to or higher in density than the net density of the fluid that hold the band of particles. This requirement for stability can be expressed mathematically as in Equation 2 and means that it is impossible to have an instantaneous, “step-like” increase in suspended particle concentration without inducing instability. In fact, there will always be some (very brief) instability immediately following sample injection until the above equation is satisfied. The effect of instability is a broader than expected initial sample band, and so lower than expected resolution.

We can estimate the effect of instability by comparing the net sample density with the steepness of the density gradient inside the disc centrifuge. For example, suppose we inject a sample of polystyrene particles with a concentration of 0.05% by weight (typical for a polystyrene sample), and that the fluid in the centrifuge ranges from 1.0178 g/ml (5% sucrose solution) to 0.9981 g/ml (water) over a sedimentation distance of 20 mm. The steepness of the gradient is:

$$(1.0178 - 0.9981) / 20 = 0.000985 \text{ (g/ml)/mm}$$

The density of polystyrene is 1.050 g/ml, so a 0.05% dispersion in water at 20°C has a density of 0.998126 g/ml, or 0.000026 g/ml higher than pure water. The distance over which this increase in density can be supported by the density gradient is:

$$0.000026 / 0.000985 = 0.0264 \text{ mm}$$

In other words, the leading edge of the sample band can not be less than 0.0264 mm wide in order to maintain stable sedimentation if the polystyrene concentration in the sample is 0.05%. The initial sample thickness (based on injected sample volume of 0.05 ml) is ~0.033 mm. Initial instability will add about 0.0264 to the initial band thickness. Higher or lower sample concentrations will lead to a proportionally larger or smaller contribution from initial instability.

Materials with higher density (for example polyvinyl chloride, density 1.385 g/ml) provoke additional instability unless a proportionally steeper density gradient is used. In nearly all cases, the effects of instability can be kept quite small by using relatively low sample concentration and an appropriate density gradient.

With 0.05% of 0.3 micron polystyrene particles, 0.1 ml sample volume, a 20 mm sedimentation distance, and the above described density gradient, the expected resolution of the instrument (including the effect of Brownian motion over 10 minutes of sedimentation) is ~1.8 - 1.9%.

- Injection Effects

The injection process can impact resolution in two ways. First, the injection is not instantaneous, but actually takes place over a period of about 0.1 second. This means that all particles do not start the sedimentation process at exactly the same time. Second, the physical impact of the sample striking the fluid surface inside the disc can cause some initial mixing of the sample into the gradient fluid, so that the initial sample band is not as narrow as the volume of the injected sample would suggest.

Injection Timing

The effect of injection timing on the reported width of a perfectly narrow family of particles depends on the total sedimentation time. The % increase in reported peak width is given by:

$$100 * ((1 + (T_i / T_s))^{0.5} - 1) \quad (\text{Eq. 7})$$

Where T_i is the time required for injection
 T_s is the time required for the particles to reach the detector beam

At a sedimentation time of 60 seconds and with an injection time of 0.1 second, the increase in width is ~0.083%. This contribution is very small compared to the other factors that impact resolution. At sedimentation times longer than about 1 minute, injection timing will always have negligible impact on resolution. With much shorter sedimentation times, the effect can be significant. For example, if a peak reaches the detector in 10 seconds, the increase in reported width from injection timing will be ~0.5% of the peak diameter; or about 25% of the total reported width.

The overall impact of injection timing is actually a little less than indicated by the above equation, because if particles begin sedimentation at slightly different times, the effect of initial instability (as described in the above section) will be reduced. Reduced initial instability partially offsets the effect of injection timing.

Physical Impact

It is difficult to predict the effect of physical impact of the sample on the fluid surface. However, experience has shown that the initial mixing (band broadening) is relatively small in nearly all cases, especially when the total sedimentation distance is ~20 mm. The rotating disc can be viewed using a synchronised strobe light, and the mixing from physical impact of the injection can be seen; it is clearly <1 mm, although an exact value is difficult to measure. When the sample is prepared using a fluid that is significantly lower in density than the fluid at the top of the density gradient, the mixing is drastically reduced (<<1 mm). For example, if the gradient consists of sucrose in water, the sample can be prepared in a mixture of 8% ethanol in water, with a density of ~0.985 g/ml. With this type of sample preparation fluid, the sample does not penetrate the density gradient surface very far; the sample fluid tends to quickly “float” and spread across the fluid surface. While it is not possible to exactly predict the effect of physical impact, the contribution to reported width of a perfectly narrow beam should be <1% in all cases, and likely will be well under 0.5% if the sample is prepared in a lower density fluid.

Actual Resolution of the DCS

When all of the factors that impact resolution are taken into account (Brownian motion, initial instability, injection effects), the expected resolution at a particle diameter of 0.3 micron is in the range of 1.9% to 2.5% when the instrument is set up with a sedimentation depth of 20 mm. This means that two perfectly narrow distributions of particles of the same material that differ in diameter by 1.9% to 2.5% should overlap by not more than 5% of their peak area.

Enhancing Resolution in the DCS

The most important factors that impact instrument resolution are known (detector beam width, sample thickness, Brownian motion) and can be mathematically modeled. It is possible to enhance the instrument’s resolution by mathematically treating the distribution data that comes from the instrument to remove the effects of these factors. The process of removing a known effect from an unknown distribution is sometimes called “deconvolution”.

Of course, for almost all measurements, the basic resolution of the DCS method is more than adequate, and deconvolution is not needed.



Sensitivity and Sample Size

The DCS method is very sensitive, especially in the size range of 0.01 μ to 10 μ diameter, where the efficiency of light scattering is high. For larger and smaller diameters, sensitivity becomes gradually lower. Broad distributions require more sample weight, but any sample with a total dry weight of 50 to 100 μg usually produces a good distribution. Some other analysis methods require much larger sample sizes.

Speed of Analysis and Dynamic Range

Total analysis time depends on centrifuge speed, particle density, fluid density, fluid viscosity, minimum particle size, maximum particle size, and data collection rate. Different commercial instruments often have large differences in total analysis time for the same sample. A higher data collection rate (more data readings per second) allows a wider dynamic range to be measured in the same total analysis time, because larger (faster moving) particles can be measured more accurately. A higher maximum centrifuge speed reduces total analysis time for samples with very small particles. Dynamic size range has a very strong effect on total analysis time. Using a constant speed centrifuge and constant detector position, and measuring a dynamic size range of 25 (ratio of largest size to be measured to smallest size in the distribution), the total analysis time will normally range from ~10 minutes to ~40 minutes, depending on the instrument. If the dynamic range is 50, then analysis time for most samples will be from ~40 to ~160 minutes, depending on instrument. If the dynamic range is relatively narrow (<15) then most samples can be analysed within ~4 to ~16 minutes.

Some types of samples contain a very wide range of particle sizes. Historically, these samples have been very difficult to measure using the differential sedimentation sizing method, because differential sedimentation has been limited to a dynamic range of about 70. A modified disc design and ramping of centrifuge speed can now be used to greatly increase dynamic range with the DCS. A dynamic range of over 1000 can easily be attained.

Dynamic Range Limit for Fixed Speed

Suppose that you had a sample where you wanted to measure particles between 20 microns and 0.05 microns. You must select a centrifuge speed so that the 20 micron particles (fastest moving) arrive at the detector beam no faster than ~0.75 second after injection, because it is not possible to accurately time the sample injection and collect distribution data with a sedimentation time below about ~0.75 second. The smallest particles will sediment at a much slower rate than the largest. The ratio of sedimentation speeds is: $(D_1 / D_2)^2$. If the 20 micron particles reach the detector in 0.75 second, then the 0.05 micron particles reach the detector at $0.75 * (20 / 0.05)^2$, or 120,000 seconds, or 33 hours and 20 minutes. This is clearly not a practical analysis time, and even if you were willing to wait 33 hours for results, Brownian motion of the smallest particles over 33 hours of sedimentation would cause substantial errors in the reported distribution.

The dynamic range depends in reality upon how long you can wait for results. If the longest run you can tolerate is 30 minutes, then the practical dynamic range is:

$$((30 * 60) / 0.75)^{0.5} = 48.99$$

If you can wait only 15 minutes, the dynamic range falls to 34.64; if you can wait 60 minutes, the practical dynamic range is 69.28.

Many types of samples (if not most types) are easily measured with a dynamic range of 30 to 70, and so present no problem for the DCS. However, there are some types of samples that really do have a broader dynamic size range, and these have previously been difficult or impossible to completely characterise using the DCS.

Ramping the Disc Speed

The G-force inside the centrifuge is proportional to the square of the rotational speed, and so the sedimentation velocity of a particle is also proportional to the square of the rotational speed. If the rotational speed at the beginning of an analysis were low, and then gradually increased during the analysis, then the problem of limited dynamic range would be resolved: the lower initial speed allows analysis of the large end of the distribution, while the higher final speed allows you to measure the smallest particles in a practical run time.

Consider the 20 microns to 0.05 micron sample discussed in the above section (dynamic range of 400). If the rotational speed were increased by a factor of 12 over the first 6 - 10 minutes of the analysis, then the whole size range could be measured in a run lasting only ~20 to 23 minutes, which is a reasonable run time for most particle sizing applications.

To make speed ramping practical, a couple of operational problems have to be resolved.

Disrupting the Gradient

Stable sedimentation (no “streaming”) in the DCS depends on having a density gradient inside the disc chamber, where the fluid at the outside edge of the disc is slightly higher in density than the fluid at the surface, and where the fluid density changes gradually between these two extremes. So long as the disc is turning at constant speed, the fluid inside the disc is “quiescent”; there is no reason for the gradient to be disrupted. If the disc speed is changed after the gradient is established, the change in speed will cause turbulent mixing of the fluids inside the disc, and total disruption of the density gradient. In other words, the fluid inside the disc will become uniform in composition and stable sedimentation will not be possible.

Calculating the Particle Size

If it were possible to avoid disruption of the density gradient, it would still be necessary to account for the changing speed so that an accurate particle size distribution can be calculated.

Resolving the Speed Ramp Problems

Figure 6 shows how the disc is modified to avoid disruption of the gradient. A “separator wall” is placed inside the disc chamber. During acceleration or deceleration of the disc, this separator keep the fluid inside the disc from moving at a speed different from the disc speed, and so eliminate virtually all mixing due to acceleration/deceleration.

The problem of calculating an accurate size distribution with changing speed is solved by modifying the operating software account for changing disc speed.

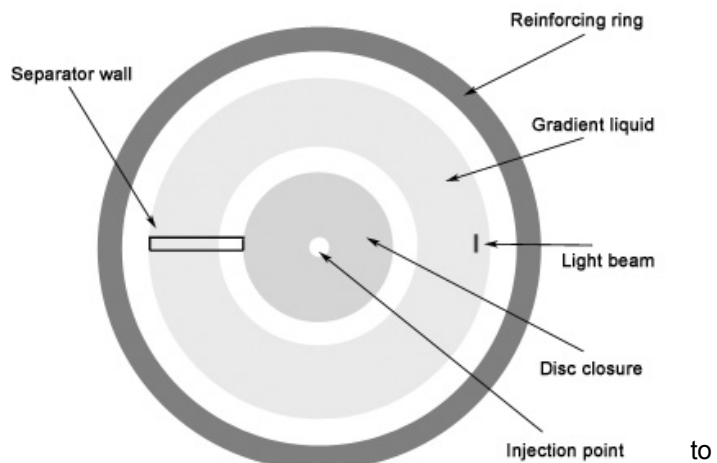


Figure 6 – Disc Design for Speed Ramping

The equation below is Stokes’ Law, modified to account for the changing g-forces inside a centrifuge:

$$D = \{ (18 \eta \ln (R_f / R_0)) / ((\rho_p - \rho_f) [\omega_t^2 d t]) \}^{0.5} \tag{Eq. 8}$$

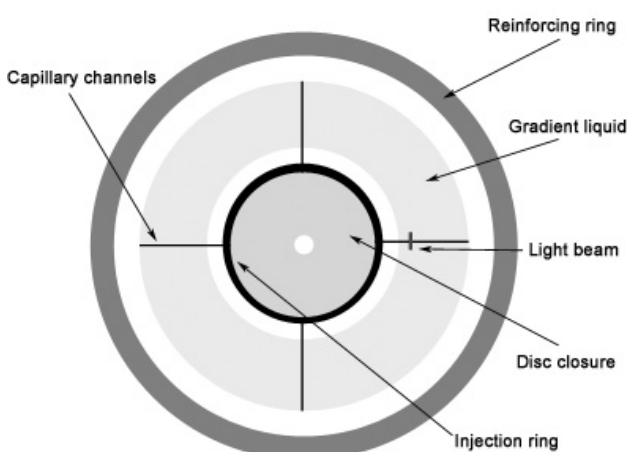
So long as we know how the rotational speed ω_t varies with time we can continuously integrate with respect to time during an analysis and generate an accurate distribution.

Low Density and Neutral Buoyancy Particles

The most important historical limitation for differential centrifugal sedimentation has been the requirement that the particles to be measured be significantly higher in density than the fluid in the centrifuge. A minimum density difference of 0.05 g/ml is desirable for most samples, and a difference of 0.1 g/ml or more is better. Some aqueous dispersions, such as polymer latexes and oil emulsions, often have particle densities near or below 1 g/cc. It is possible to use a mixture of water and methanol or ethanol, which has a density lower than water, to measure some types of low density samples, but many are not compatible with the required alcohol concentration. Many low density dispersions have historically been impossible to measure using the DCS method.

A New Differential Method

A new method⁷ has been developed for differential sedimentation of low density materials. The new method uses a centrifuge design that deposits a low density sample at the bottom of a spinning centrifuge chamber, rather than at the surface of the fluid in the chamber. This method requires that the particles be lower in density than the fluid in which they are suspended; the particles move from the bottom of the chamber toward the top during the analysis. The implementation of the new method in a centrifuge of the hollow disc design is shown in Figure 7. A "V" shaped groove is machined into the front face of the hollow disc, and four or more small capillary channels go radially from the base of the "V" groove to connect with the bottom of the centrifuge chamber. The level of the base of the "V" must be at least slightly above the level of fluid in the centrifuge (a lesser distance from the center of rotation) to keep the groove free of liquid.



A sample is injected into the groove at the start of an analysis. Typical injection volume is in the range of 20 to 50 μ litres. When a sample is injected into the "V" shaped groove, it is quickly (<0.1 second) carried by centrifugal force to the bottom of the centrifuge chamber via the small radial channels. The combined volume of the channels can be less than 10 μ litres, so even a small sample volume is sufficient to displace the liquid in the channels.

Figure 7 – Modified Disc Design for New Method

Any sample that remains in the channels may be flushed to the bottom of the centrifuge by immediately following the sample with a small volume (10 to 20 μ litres) of the same fluid that was used to prepare the sample for injection.

The large central opening to the disc chamber may be covered with a removable insert. With the insert in place, density gradient fluids and samples can be injected directly onto the center of the rotating disc, rather than into the "V" shaped groove. Rotation of the disc quickly carries any injected fluid or sample to the base of the "V" shaped groove, and then to the bottom of the chamber via the capillary channels. When a removable insert is used in the center of the disc, the density gradient may be formed by injecting a series of fluids with slightly different densities; the lowest density fluid first and the highest density last. The lower density fluids float upon the higher density fluids, and there is only a small amount of mixing as the gradient is formed.

Samples are prepared for analysis by dilution in a fluid which is more dense than the fluid at the bottom of the centrifuge chamber. The net density of the sample dispersion (average of particles and fluid) must be higher than the density of the fluid at the bottom of the centrifuge chamber, so that the dispersion of particles quickly spreads to form a thin layer at the bottom of the chamber.

Sedimentation of the particles proceeds in the normal fashion, except that the particles move toward the surface of the fluid rather than toward the bottom of the centrifuge chamber. Multiple analyses can be run without stopping the centrifuge, and it is even possible to alternate analyses between samples that are higher in density than the fluid, which are injected onto the surface, and samples that are lower in density than the fluid, which are injected into the "V" shaped groove.

By using either the conventional differential method or the new differential method reported here, virtually any sample which is an aqueous dispersion can be measured by differential sedimentation. If the particles are significantly higher in density than water, they can be analysed using the conventional differential method. If the particles are significantly lower in density than water, they can be analysed using the new method with water in the centrifuge. If the particles are near the density of water, then the new method can be used with deuterium oxide partially or totally substituted for water in the centrifuge.

The new differential method can be extended to centrifuges of nearly any design, and to many non-aqueous solvent systems as well, so long as the fluid within the centrifuge has a density gradient and so long as the samples are prepared in a fluid that is both higher in density than the fluid at the bottom of the chamber and miscible with the fluid at the bottom of the centrifuge chamber. A sample may be prepared in a fluid that is not miscible with the fluid at the bottom of the centrifuge chamber, so long as the interfacial surface tension between the fluid phases does not prevent particles from passing from one phase to the other.

Eliminating Injection Artifacts

The new differential method yields distributions that may include small injection artifacts. These artifacts have no connection to the actual particle size distribution; they are seen even when a blank (particle free) sample is analysed. The injection artifacts are of two types:

- relatively large diameter particles that are actually air bubbles entrained when a sample is injected;
- a relatively broad baseline deflection that comes from a slight change in optical density of the fluid in the centrifuge when a sample is injected.

The injection artifacts can be minimised or eliminated using one or more of the techniques discussed below.

Entrained air bubbles show up as large "particles" because they rise rapidly through the fluid: they are both relatively large in size and much lower in density than the fluid in the centrifuge. The volume of entrained air bubbles can be minimised by having the level of the fluid within the centrifuge close to the top of the capillary channels that transport samples to the bottom of the centrifuge (please refer to Figure 7), and by using capillary channels that are of the smallest practical diameter. If the distance between the top of the fluid and the top of the capillary channel is small and the diameter of the capillary channel is also small, then the artifact from entrained air bubbles is minimised.

The mechanism for production of the second type of injection artifact (that due to a change in optical density of the fluid) is not obvious. Before a sample is injected, the fluid in the centrifuge chamber is moving at the same rotational speed as the centrifuge. When a small sample is injected at the bottom of the centrifuge chamber, the total volume of the fluid in the chamber increases very slightly. All of the fluid in the chamber is raised slightly when a sample is injected, because the sample is higher in density than the fluid in the chamber and enters at the bottom of the chamber. When the fluid is raised, it rotates at a very slightly smaller radius than it rotated before the injection. The absolute linear velocity of all of the fluid in the chamber is not immediately changed when a sample is injected, but the radius of rotation for the fluid in the chamber is suddenly (very slightly) reduced when a sample is injected. This means that the rotational velocity of all of the fluid in the chamber increases slightly relative to the rotational speed of the centrifuge at the moment a sample is injected. The physical effect of the injection is similar to a small, instantaneous, reduction in centrifuge speed. Inside a hollow disc type



centrifuge, the fluid cannot suddenly change in speed, it must gradually catch up with the speed of the centrifuge disc.

This difference in speed between the centrifuge and fluid causes slight mixing to take place within the fluid as its rotational velocity recovers to match the rotational velocity of the centrifuge. The fluid in the chamber is not uniform in composition; its composition changes due to the presence of the density gradient. If the refractive index of the fluid also changes as the composition of density gradient changes, then mixing caused by injection of a sample will cause some of the detector light beam to be scattered: the optical transmission of the fluid in the chamber is slightly reduced due to optical inhomogeneity during mixing. As the homogeneity of the fluid gradually recovers (due to diffusion), the optical transmission returns to the original level.

The artifact due to changing rotational speed can be minimised in two ways. First, the smallest practical sample volume can be used. The smaller the sample volume (relative to the volume of the centrifuge chamber) the smaller the effect of the injection. Second, a density gradient can be prepared that is constant in refractive index. If all of the fluid in the chamber has the same refractive index (even though the composition does change), then mixing will not cause the optical transmission of the fluid to change. Density gradients with virtually constant refractive index can be formed using mixtures of three components. For example, an aqueous density gradient that goes from 2% to 0% (by weight) sucrose and at the same time from 0% to 5% ethanol has nearly constant refractive index over the entire composition range.

Both types of injection artifact can be mathematically subtracted from a particle size distribution. A blank sample (free of particles) can be run to record only the injection artifacts, and then subtracted from the distribution of an unknown sample. The distribution that remains after the subtraction is the distribution for the unknown, free of injection artifacts.

Conclusion Regards Low Density and Neutral Buoyancy Particles

By using the new method reported here, differential centrifugal sedimentation can be applied to measure the size distributions of materials that are lower in density than the fluid in which they are suspended. This eliminates the single most important limitation of the differential method, while maintaining the high resolution, accuracy, and operational advantages of the differential method.

Non-spherical Particles

The weight distribution reported by the DCS method is a "Stokes-equivalent" distribution: the weight distribution of spherical particles that would yield the reported distribution. The Stokes-equivalent distribution is equal to the true weight distribution only if the particles in the distribution are spherical. Particles with other geometries are reported somewhat smaller than their actual weight distribution. For particles that closely approximate spheres (for example icosahedrons), the measured distribution will be very nearly correct, while geometries very different from spheres, like long thin rods, will be reported as significantly smaller than their actual weight distribution.

Cylindrical rods with an aspect ratio of ~ 2 (length/width) produce a reported weight distribution about 5% smaller than correct, while rods with an aspect ratio of ~ 3 produce a reported weight distribution about 10% smaller than correct. Particles with a disk shape, ~ 2 times wider than they are thick, are reported as about 6% smaller than correct. For all non-spherical particles, no matter what the geometry, the DCS method produces very consistent and repeatable results, even if those results are not exactly correct in absolute weight sense. The DCS method is commonly used for characterisation and quality control with a wide range of inorganic pigments, fillers, and abrasives, even though the particles being measured are not spherical in shape.

Future Trends

Developments in DCS over the next five years are probable in three areas.

1. Overall instrument sensitivity and dynamic signal range will continue to improve, due to higher analog S/N ratio, higher resolution analog to digital conversion, and improved (software based) noise filtration. Sensitivity and dynamic signal range will likely improve by at least a factor of 5. An analysis that today requires at least 1µg dry sample weight will probably require ~0.2µg within the next 5 years. Improved sensitivity will allow analysis of near trace quantities of particulate contaminants in liquids. Contamination of one particle size with another (for example, 0.40µ particles contaminating a sample of 0.50µ particles) should be detectable at less than 1 part in 5,000.
2. Instrument resolution will continue to improve, due to better detector beam optics and optimized data deconvolution. Routine resolution of particles that differ in size by <2% should be possible.
3. Completely automatic on-line DCS systems will be developed if there is sufficient need for high resolution on-line measurement of particle size distributions in the 0.02µ to 30µ size range. A self-contained automated system (similar to on-line gas chromatographs) could be attached to the product stream from a continuous or batch process, and automatically sample, measure, and report particle size distributions at almost any desired frequency.

Introduction to Differential Sedimentation – Overall Conclusion

Differential Centrifugal Sedimentation is an extremely powerful tool for high resolution particle characterisation, especially in the size range 0.003 micron (3 nm) to 10 micron. It enables very narrow distributions of particles differing in diameter by less than 2% to be resolved and hence extremely small differences, changes or shifts in particle size to be accurately and reproducibly detected and measured. The new method described in this article for measurement of low density neutral buoyancy particles also addresses the only previous technical benefit of integral sedimentation over DCS.

The DCS instrumentation used for this article is the CPS Disc Centrifuge DC24000.

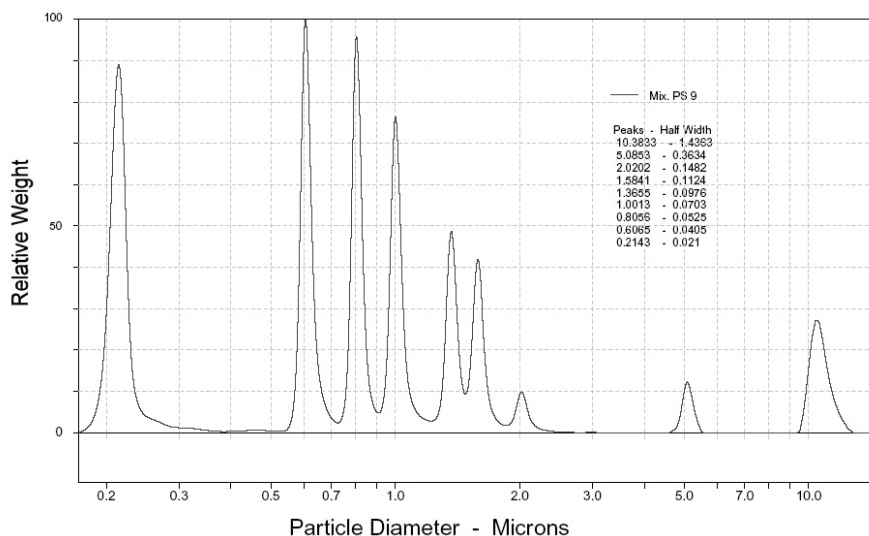


Figure 8 – Resolution of multi-modal distribution of polystyrene particles



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