ANALYSIS OF LOW DENSITY PARTICLES USING DIFFERENTIAL CENTRIFUGAL SEDIMENTATION

Conventional Centrifugal Methods

Centrifugal sedimentation of particles suspended in a fluid is a well known method (1, 2) to measure the size distribution of particles in the range of about 0.02 micron to about 20 microns. The sedimentation velocity of any particle can be calculated if the particle density, fluid density, fluid viscosity, and centrifugal acceleration are known, using the well known Stokes' Equation (3). Stokes' Equation is modified slightly to account for how the centrifugal force inside a centrifuge changes as the radius of rotation increases, since during an analysis the radius of rotation changes as particles sediment. There are two conventional techniques for analysis: **integral** (sometimes called homogeneous) sedimentation and **differential** (sometimes called two-layer) sedimentation. These two techniques have inherent advantages and disadvantages.

In the less commonly used technique, integral sedimentation, each analysis starts with a homogeneous suspension of particles within a centrifuge; particles sediment out of the suspension at rates that depend upon particle size. The concentration of particles remaining in the suspension is measured during the analysis, usually with a light beam or x-ray beam that passes through the centrifuge. The result of the integral analysis method is a cumulative representation of the particle size distribution. The integral technique has several operational disadvantages, the most important of which are inaccuracy due to rapidly changing (and difficult to characterize) conditions at the start of the analysis, possible inaccuracy due to thermal convection within the sample during the analysis, and the need to stop, empty, and clean the centrifuge after each sample. The single important advantage of the integral method is the ability to measure particles that are either higher or lower in density than the fluid in which they are suspended. Particles that are higher in density than the fluid sediment toward the bottom of the centrifuge (that is, toward the point furthest from the center of rotation) while particles lower in density than the fluid sediment (float) toward the top of the centrifuge (that is, the point closest to the center of rotation).

In the more commonly used technique, differential sedimentation, a small sample of particles is placed on top of clear fluid and subjected to centrifugal acceleration. (Figure 1). Particles sediment at velocities that depend upon size, until reaching the detector beam (light or x-ray) that passes through the fluid at a known distance below the fluid surface. The concentration of particles in the path of the detector beam is initially zero, and changes during the analysis depending upon the distribution of sizes in the sample. All particles start sedimentation at the same distance from the detector beam, and at the same time. Particle size is calculated from arrival time at the detector. The result of the analysis is a differential particle size distribution. An integral distribution may be generated by integrating the differential distribution with respect to particle size.

In actual practice, differential sedimentation requires a slight density gradient within the fluid inside the centrifuge to insure that no instability develops during sedimentation. This instability is sometimes called "streaming". Streaming is settling of the sample as a bulk liquid, rather than as individual particles, and is due to the effect of the suspended particles on the overall density of the fluid in which they are suspended. If the net density of the fluid that contains the sample (particles plus fluid) is greater than the density of the fluid immediately below, then the sedimentation process may become unstable. Stability is assured if the following condition is satisfied:

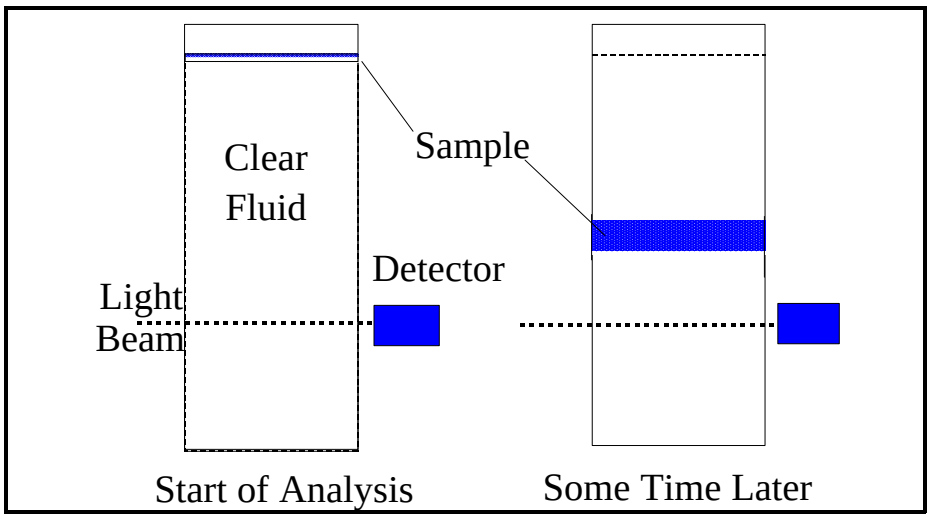

Figure 1 - Differential Sedimentation Method

$$\frac{d\tilde{n}}{dR} \geq 0$$

Where ñ is net fluid density, including particles
R is the distance from the center of rotation

The density gradient in the centrifuge can be quite small; an increase of 0.01 g/cc or less per centimeter of fluid height usually sufficient to insure stable sedimentation, so long as the concentration of particles in the sample is low. The effect of the density gradient on sedimentation speed is small so long as the particles are significantly more dense than the fluid. The presence of a density gradient also eliminates thermal convection currents in the fluid within the centrifuge, so the sedimentation analysis is not disrupted by slight temperature changes during the analysis.

Samples are prepared for analysis by dilution in a fluid which is lower in density than the fluid at the top of the centrifuge column. This causes samples to spread over the surface of the fluid in the centrifuge, so that all particles in each sample begin analysis at the same distance from the detector beam.

For aqueous fluids, sucrose is usually a good choice to form a gradient, but a wide variety of water soluble compounds may be used. A suitable gradient can be produced manually, by sequential addition of fluids to the centrifuge in order of decreasing density, by an automatic gradient producing machine, or by other methods (4,5).

The design of the centrifuge varies depending on manufacturer, but the most common

design is based upon an optically clear rotating hollow disc (Figure 2). The hollow disc is driven by a motor which can be set to run over a wide range of speeds, so that a wide range of particle sizes can be measured with the same instrument. Prepared samples are injected into the center of the rotating disc at the start of an analysis; rotation of the disc carries the sample to the surface of the fluid.

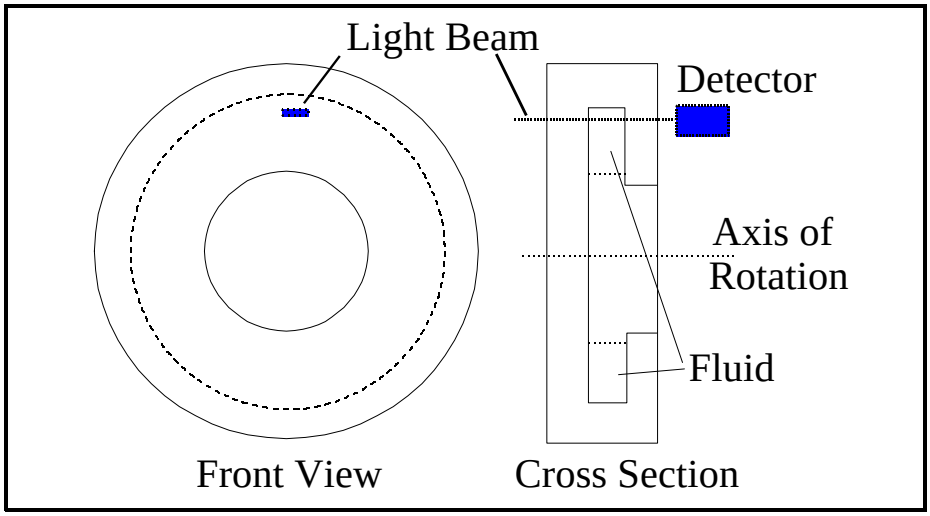

Figure 2 - Hollow Disc Centrifuge Design

Differential centrifugal analysis normally yields very high resolution, accurate, and reproducible particle size distributions. The principle advantage of the differential method over the integral method is that many samples may be run in series without having to stop and empty the centrifuge. The run duration is limited only by loss of the density gradient with time. The gradient is gradually lost by molecular diffusion of the components that form the gradient, but may also be lost due to physical mixing of the fluid in the centrifuge. Physical mixing can be caused by evaporation at the surface of the fluid, or by small changes in the rotational speed of the centrifuge during operation. By limiting evaporative losses and by careful control of the rotational speed, it is possible to extend operation of the centrifuge to at least several hours, and many different samples can be analyzed during that time. Continuous operation reduces the overall time needed to run an analysis, reduces operator labor, and makes automation of sample injection and data collection straightforward.

The most important disadvantage of the differential method is the requirement that the particles be more dense than the fluid in which they are suspended. This requirement makes analysis of many low density particles difficult or impossible. Some examples of difficult samples are: butadiene styrene copolymer latexes (densities of 0.92 to 1.03, depending on monomer ratio), polybutadiene latexes (0.89 to 0.90 g/cc), ground polyethylene resins (0.91 to 0.96 g/cc), nitrile rubber latexes, acrylic adhesive latexes, and many others.

A New Differential Method

A new method (6) 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 3. The cross-section of the centrifuge disc shows how samples are transported to the bottom of the hollow disc centrifuge chamber. 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 liters.

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 liters, so even a small sample volume is sufficient to displace the liquid in the channels. 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 liters) of the same fluid that was used to prepare the sample for injection.

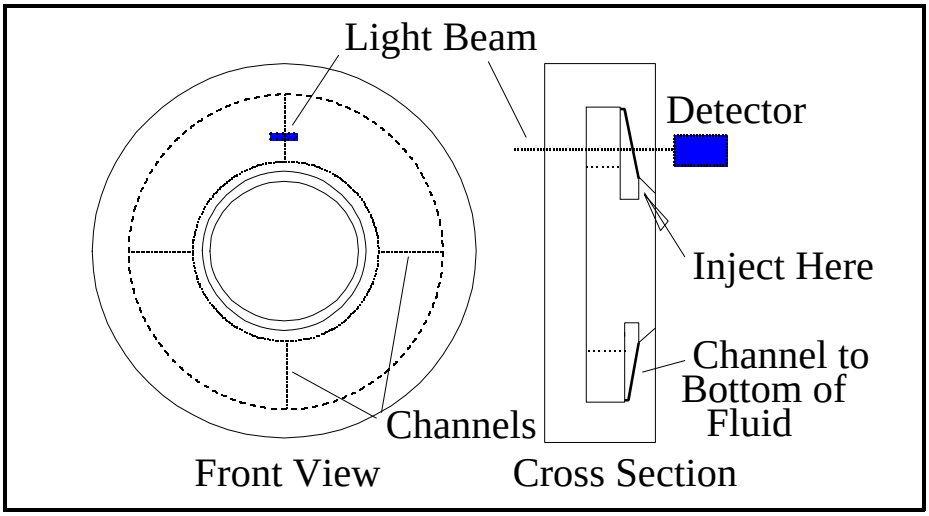

Figure 3 - Modified Disc Design for New Method

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.

Figure 4 shows duplicate analyses of a polybutadiene latex (polymer density of 0.89 g/cc) that were run using the new method. A density gradient was produced by filling the centrifuge with a series of sucrose in water solutions (4% to 0%, 1.0139 g/cc to 0.9989 g/cc). The total fluid height in the centrifuge was about 1 cm, and a detector beam (430 nanometer light) passed through the disc at 6 millimeters from the outside of the centrifuge chamber. The sample was prepared for injection by dilution to 0.2% active in an aqueous solution of 6% sucrose and 0.05% sodium lauryl sulfate emulsifier. The net density of the prepared sample was 1.0216 g/cc, and 50 µ liters were injected for each analysis. The disc speed was 8,600 RPM and the analysis time (to 0.15 micron) was approximately 17 minutes.

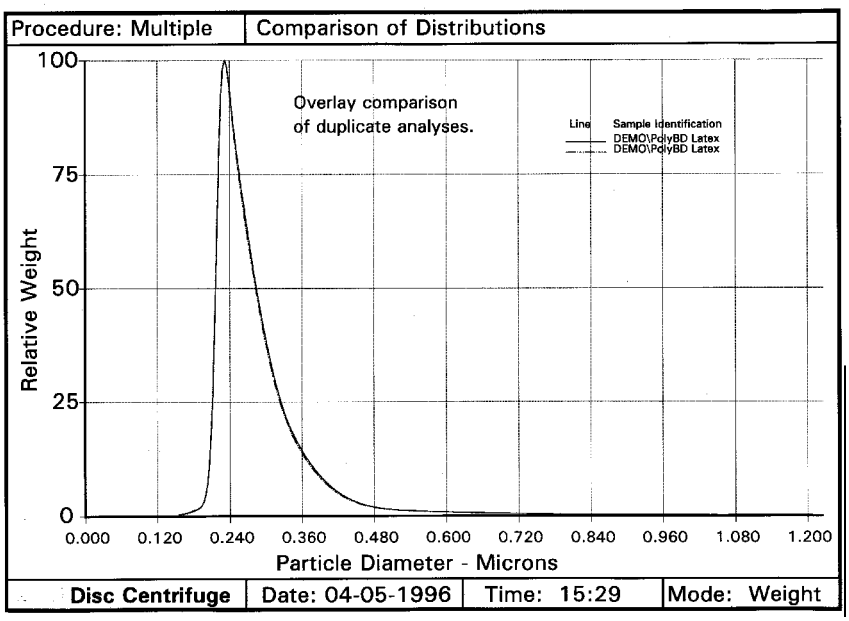

Figure 4 - Replicate Analyses of a Polybutadiene Latex

The new method can be easily extended to measure size distributions for particles in aqueous suspension with densities ranging from slightly below the density of water to slightly above the density of water. Figure 5 shows an overlay comparison of three replicate analyses of a narrow, 0.40 micron polystyrene latex. The density of polystyrene (1.050 g/cc) precludes analysis by this new method in an aqueous system, because the particles are more dense than water. However, these analyses were run using deuterium oxide (>99% D_2O, 1.107 g/cc) in place of water, to provide the required buoyancy for the analysis. A density gradient was produced by filling the centrifuge with a series of sucrose in deuterium oxide solutions that ranged from 4% to 0% sucrose. The sample dilution contained 6% sucrose (in deuterium oxide), and 0.03% polystyrene, to give a

prepared sample dispersion with an overall density of about 1.137 g/cc. A diluted sample of 30 µ liters was injected in each analysis. The centrifuge speed was 8,600 RPM, and the analysis time (to 0.25 micron) was 10 minutes. Analysis of particles with a density of 1.00 g/cc in deuterium oxide is significantly faster than polystyrene because the net buoyancy of 1.00 g/cc particles in deuterium oxide is higher than polystyrene. The ability to measure polystyrene particles using the new method allows the many widely available polystyrene latex calibration standards to be used to verify the accuracy of analyses. The near perfect replication of the two analyses in Figure 4 and the three analyses in Figure 5 demonstrate the excellent repeatability of the new method. The narrow peak widths in Figure 5 show that the resolving power of the new method is quite good.

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 analyzed using the conventional differential method. If the particles are significantly lower in density than water, they can be analyzed 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.

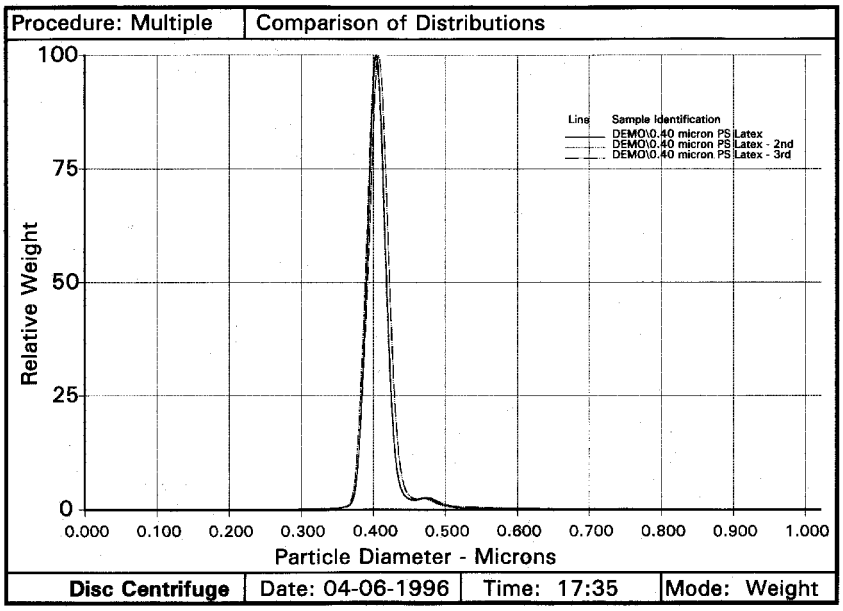

Figure 5 - Replicate Analyses of a Polystyrene Latex

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 the actual particle size distribution; they are seen even when a blank (particle free) sample is analyzed. The injection artifacts are of two types: 1) relatively large diameter particles that are actually air bubbles entrained when a sample is injected, and 2) 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 minimized 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 minimized 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 3), 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 minimized.

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 minimized 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. Mathematical removal of artifacts is shown in Figures 6 through 8. Figure 6 is the distribution for a sample blank, which shows only the injection artifact. Figure 7 is the original distribution (including artifacts) for a mixture of three narrow polystyrene calibration standards. Figure 8 is the distribution for the mixture of calibration standards after the injection artifact was subtracted (Figure 7 minus Figure 6).

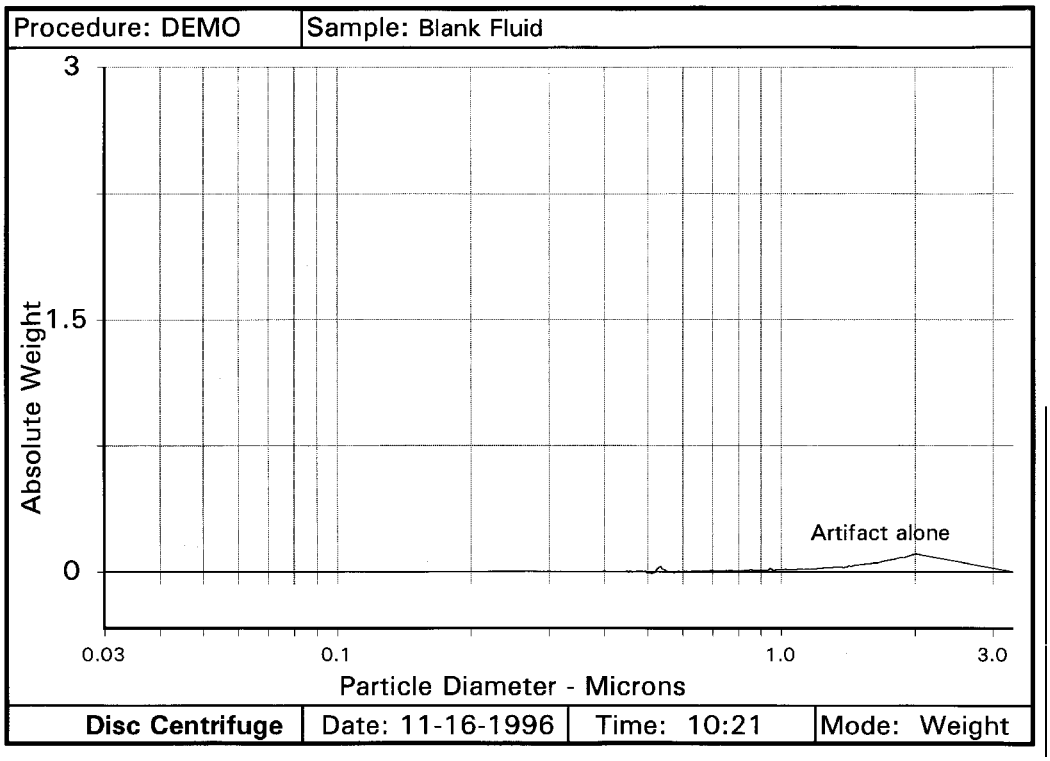

Figure 6

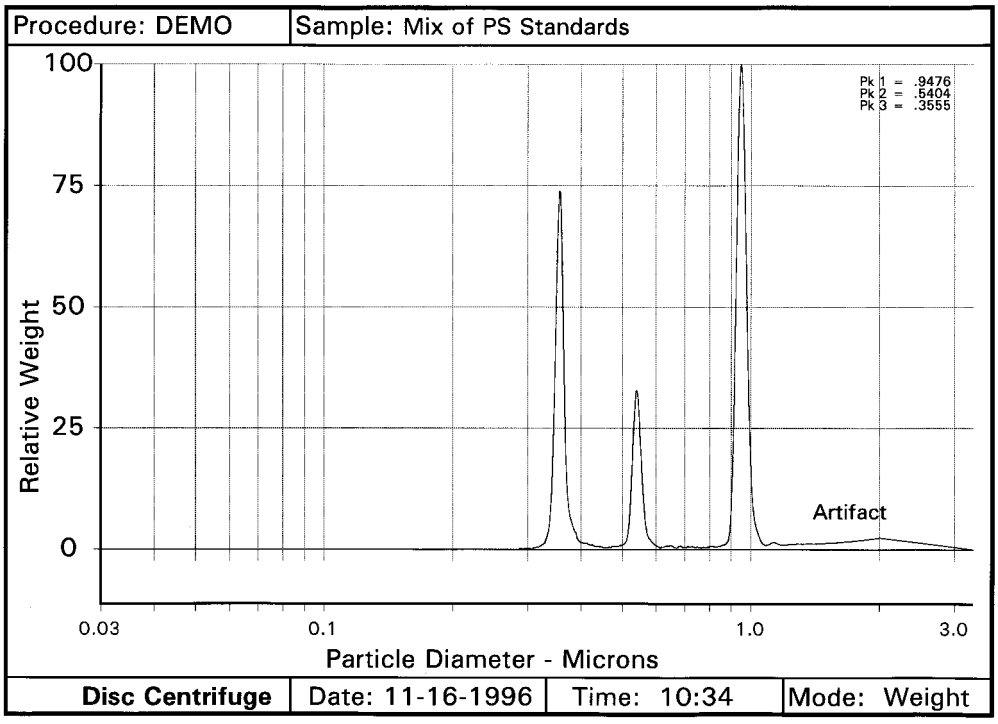

Figure 7

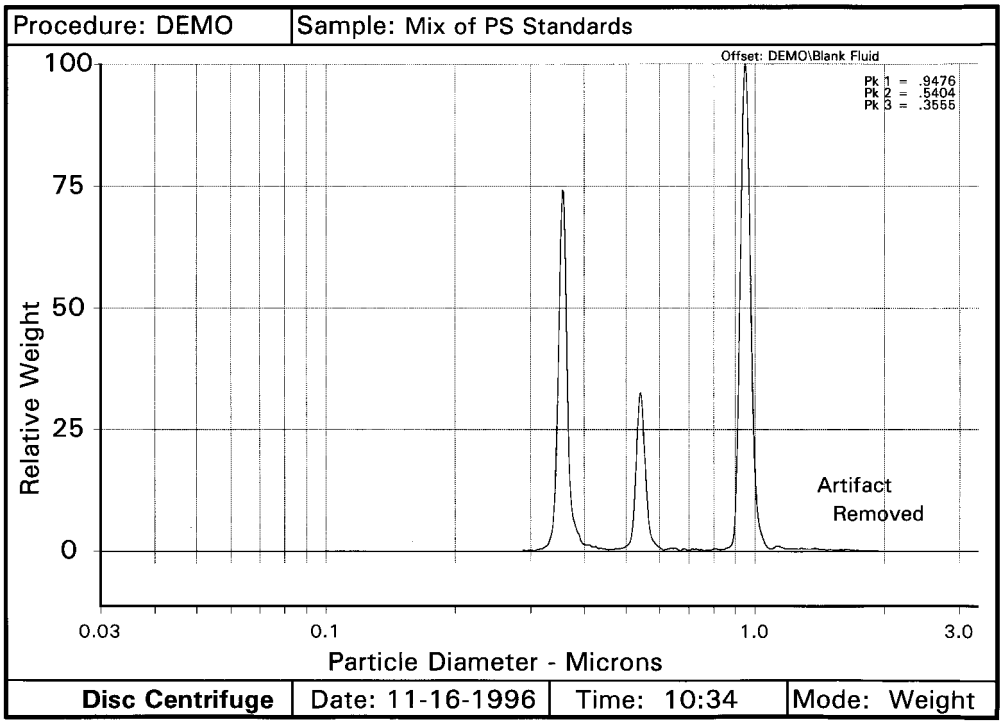

Figure 8

Conclusion

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 operation advantages of the differential method.

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