

An introduction to particle size characterisation by DCS:

Do you know the real size of your nano particles?

By Dr Hiran Vegad, Analytik Ltd

Introduction

Differential centrifugal sedimentation (DCS) is a novel and innovative, yet simple technique, which has become 'reborn' in recent years. Previous limitations and difficulties with the technique of sedimentation have been overcome using recent advances in technology, and some smart thinking regards instrumentation and disc design. DCS is now a powerful tool in measuring nano particle size distributions down to around 2nm. With the unique ability to resolve very close multi-modal particle distributions, and to distinguish extremely small shifts and changes in particle size, DCS is once more gaining in popularity. The practical range of the technique is from around 2nm right up to 80 micron (exact range will be dependent on density), however the real benefits over and above more traditional so-called nano particle sizing techniques are generally noticed below around 300nm.

These days, DCS is highly accurate, reproducible, fast, very simple to use, can measure up to 40 samples on the same 'run', does 'speed ramping' for measurement of broad distributions, and can even measure 'buoyant' or 'neutral density' particles, i.e. particles having a lower density to the medium in which they are dispersed.

Benefits of DCS

There are a number of distinct benefits associated with the DCS technique; these will be illustrated in the following section:

- *Ultra High Resolution Capability* – detect, measure & resolve peaks which differ in size by as little as 2% (Fig. 1)

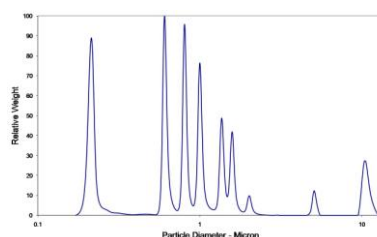


Fig. 1

- *High Sensitivity* - detect & measure extremely small and subtle changes in size distributions e.g. small additional peaks (Fig. 2)

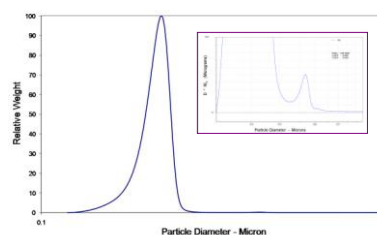


Fig. 2

- *Accurate, Reliable & Reproducible Results* – traceability & calibration to NIST particle size standards (Fig. 3)

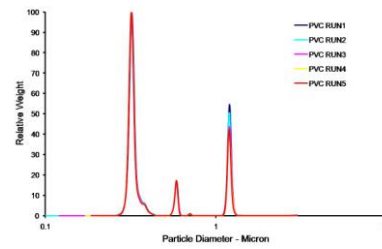


Fig. 3

- *Results that are Comparable to SEM / Microscopy Analysis* (Fig. 4)

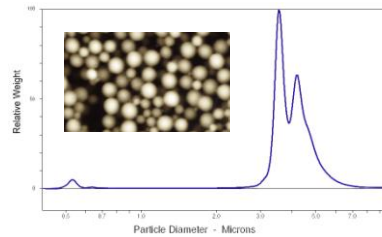


Fig. 4

- *Quantitative Analysis to give Weight & Number Distribution* (Fig. 5)

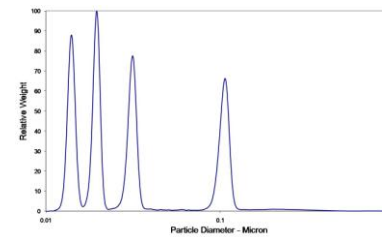


Fig. 5

Theory of Differential Centrifugal Sedimentation

Sedimentation of particles in a fluid has long been used to characterise particle size distribution. Stokes' law 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). For a centrifuge running at constant speed and temperature, all of the parameters in the equation 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, a modified form of Stokes' law accurately measures the diameter of spherical particles based on their arrival time at the detector. Hence by introducing a known, traceable standard, the time scale can be calibrated to particle size.

$$V = D^2 (\rho_p - \rho_f) G / 18 \eta$$

- D the particle diameter (cm)
- ρ_p particle density (g/ml)
- ρ_f the fluid density (g/ml)
- G the gravitational acceleration (cm/sec²)
- η the fluid viscosity (poise)

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 6. The detector beam is usually monochromatic light of relatively short wavelength (400 nm - 500 nm); as this gives better detector sensitivity when particles smaller than 100 nm are measured.

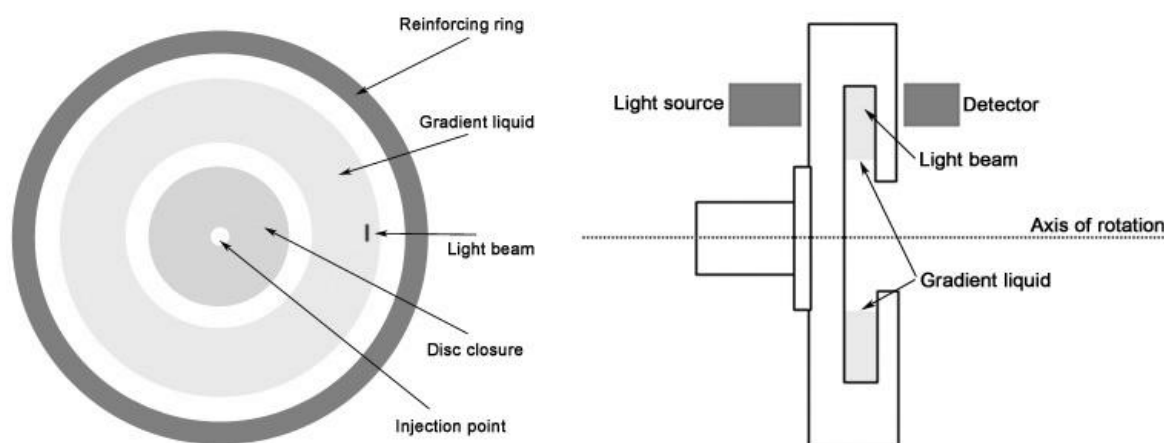


Fig. 6

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. When a sample is injected (normally around 100 μ l 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 gradient liquid. Once a sample reaches the fluid surface, sedimentation of individual particles begins. The injection of a sample is rapid (typically <50ms), so the starting time for an analysis is well defined, and the precision of sedimentation time is very good. 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.

Advances in modern instrument design have enabled compact, desktop disc centrifuges capable of 24,000rpm. This enables very short analysis times for even very small nano particles; for example, the adenovirus data shown in Fig. 9 and Fig. 10 was measured in less than 10 minutes for each sample.

Special discs and techniques are now available to enable ramping of a disc centrifuges speed during an analysis. This is useful if, for example, a sample contains relatively large particles as well as nano particles and both need to be measured. An initial slow centrifuge speed can be set to measure the larger particles; the speed is then ramped up so that the nano particles can still be measured within a reasonable timeframe.

Discs have now also been developed to enable previously very difficult analysis. For example, where particles have a lower density than the medium in which they are dispersed, they have a tendency to float rather than sediment. Special low density discs, combined with reversal of the detector position, can now enable these types of samples to be measured with ease.

Figure 7 shows a centrifuge disc inside an instrument and the light source-detector towards the outside of the disc. Figure 8 shows the same disc in rotation during an analysis, the separated bands of differently sized particles can clearly be seen as they approach the detector.

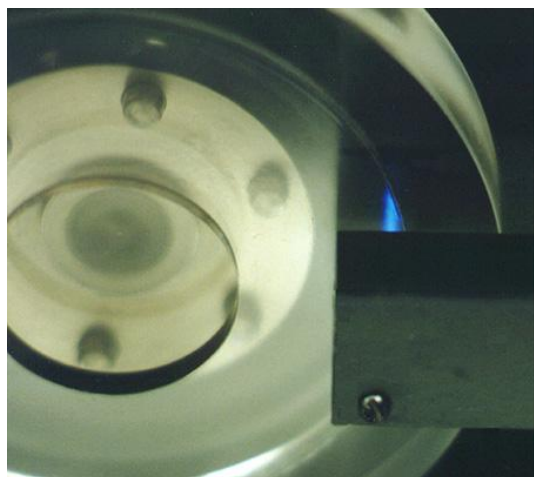


Fig. 7

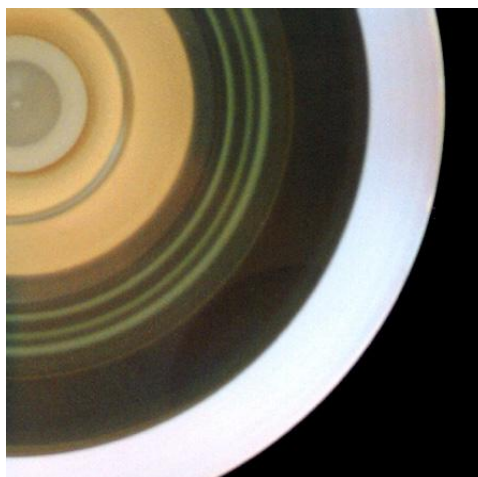


Fig. 8

Operational

Firstly an appropriate density gradient is built inside the already rotating centrifuge disc. The gradient liquid used must be compatible with the liquid in which the sample is dispersed. The most common and simple gradient to construct would be of sucrose solution, if the sample is in a buffer then a sucrose solution made up in the same buffer would be most appropriate. Organic solvents, mineral and vegetable oils can also be used in the case of non-aqueous sample systems.

Next, a calibration standard of known size and density (e.g. NIST traceable mono-dispersed polystyrene beads) is then injected to calibrate the time axis to particle size. The system is now ready for sample injections!

Normally around 100µl of each sample is injected. Up to 40X 100µl injections can normally be made before the disc becomes full and the whole process needs to be repeated with a new gradient. Autosamplers, similar to those used for liquid chromatography, can also be incorporated with a DCS system. Density gradients can be stable for anything up to 72 hours, hence very often an instrument can be set up and calibrated at the beginning of a working day and left running with calibration standards and samples being injected as and when required.

In summary, typical routine operation of a DCS system is simply:

1. Set the centrifuge at the correct speed, based on particle size and particle density (usually by retrieving an existing preset methodology from the software)
2. Fill the centrifuge chamber with appropriate density gradient fluid
3. Calibrate the instrument by running a calibration standard
4. Run samples

Prominent Applications

There are a number of applications that benefit particularly well from the DCS technique, these include:

- *Resolve Aggregates and Agglomerates* – A mixture of single adenovirus particles (Fig. 9) and multiple aggregations of the same adenovirus particles (Fig. 10) can be easily resolved as can be seen in these examples.

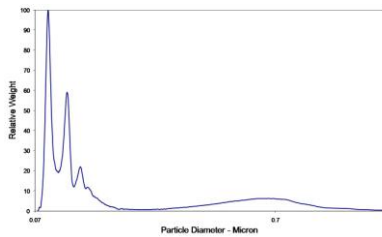


Fig. 9

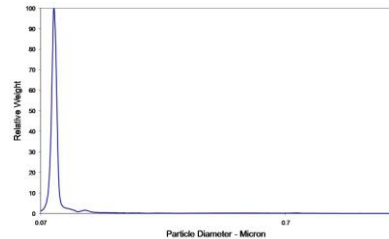


Fig. 10

- *Characterise Coating of Nano-particles* – Ability to measure/monitor coating thickness (Fig. 11)

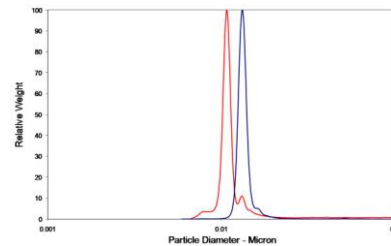


Fig. 11

- *Determine pore size distribution* – Able to test a 0.4 micron filter with a latex 'Multistandard' (Fig. 12)

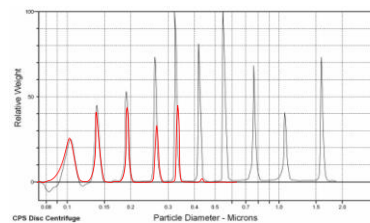


Fig. 12

- *Follow cell disruption experiments* (Fig. 13)

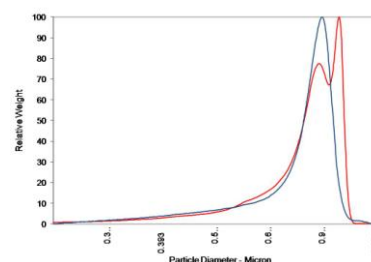


Fig. 13

Typical Applications

There are a wide range of applications for which the DCS technique can be applied; here are a few of the more typical:

Pharmaceutical and Biological:

- *Virus and virus-like particles*
- *Cells and cell fragments (culture)*
- *Protein clusters (Fig. 14)*
- *Liposomes*
- *Micro encapsulated drugs*

Chemical:

- *Polymer latexes and emulsions*
- *SiO₂ dispersions (Fig. 15)*
- *Fillers (CaCO₃, clay, barites, etc.)*
- *Abrasives of all types (Diamond - Fig.16)*
- *Carbon Nanotubes*
- *Quantum dots (Fig. 17)*

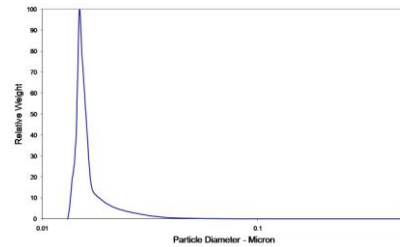


Fig. 14

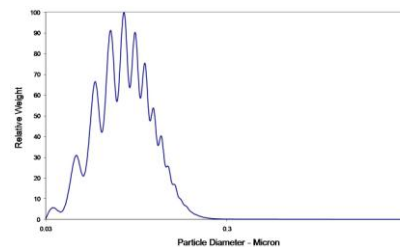


Fig. 15

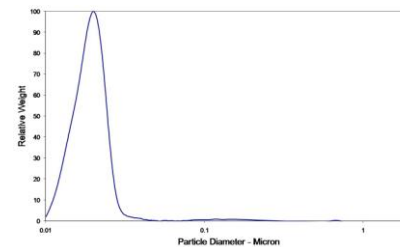


Fig. 16

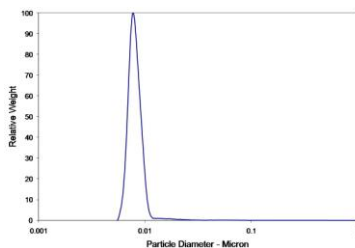


Fig. 17

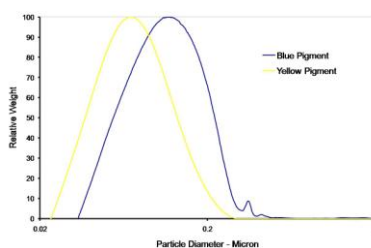


Fig. 18

Printing and painting:

- *Pigments - water and oil based*
- *Micro-fiber paint viscosity modifiers*
- *Printer/copier toner powders*
- *Inkjet inks. Fig. 18 shows two overlaid particle size distributions on yellow and blue inkjet pigments. Note the small resolved peak on the leading edge of the blue pigment which could be significant regards possible jet clogging problems.*
- *Carbon black*
- *Magnetic iron oxide*

DCS - An Overall Conclusion

Differential Centrifugal Sedimentation is an extremely powerful tool for high resolution particle characterisation, especially in the size range 0.002 micron (2nm) to 10 micron. It enables very narrow distributions of particles differing in size 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 mentioned in this article for measurement of low density, neutral buoyancy particles, addresses the only previous technical limitation of DCS. Advances in recent instrumentation, have also overcome previous issues with the technique with respect to ease of use, speed of analysis, accuracy and multiple sample measurement.

The DCS instrumentation used for this article was a *CPS Disc Centrifuge model DC24000* (Fig. 19).



Fig. 19

To learn more about high-resolution particle size characterisation using the CPS Disc Centrifuge please visit www.analytik.co.uk (UK and Ireland) or alternatively visit www.cpsinstruments.eu.

Article by:

Dr Hiran Vegad – CPS DC Specialist at Analytik Ltd
Barn B, 2 Cygnus Business Park, Middle Watch, Swavesey, Cambridge, CB24 4AA
Tel: 0870 991 4044
Email: info@analytik.co.uk

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