Equipment Name: Differential Centrifugal Sedimentation (DCS) or analytical centrifugation	**Category:** **A. Particle synthesis (characterisation)** **C. Particle Characterisation in and ex-situ** **Institute:** University College Dublin **Location:** Centre for BioNano Interactions, Conway Institute, University College Dublin, Belfield, Dublin 4, Ireland **Contact Details of Technology Expert:** **Name:** Dr. Marco Monopoli **Phone:** +353 1 716 2418 **Fax:** **E-mail:** marco.monopoli@cbni.ucd.ie

Short technology description/Overview:

Differential Centrifugal Sedimentation (DSC) or analytical centrifugation is suitable for high resolution particle size characterisation of virtually any material from 0.003 micron (3nm) to 50 micron (depending on the particle density). Particle size distributions are measured using a spinning disc with a sucrose gradient to separate particles on the basis of size, and the system can routinely separate particles that differ in size by as little as 2-5%, including in complex solutions such as plasma or cell culture media. Unlike most other particle sizing techniques, particles are actually separated and then measured, so no predictive algorithms are used.

The results from a DSC show almost "chromatographic" type resolution which is in dramatic contrast with other particle sizing techniques e.g. light scattering methods. The ultra-high resolution makes this an ideal method for effectively resolving aggregates and agglomerates, and for observing relative shifts in peaks and tails on particle distribution charts that are not clearly visible with other techniques.

UCD have developed protocols for the study of nanoparticles effective size *in situ* in complex biofluids, which can be made available through QNano TA. Applicability of these protocols to other biofluids, such as river water containing natural organic matter and nanoparticles dispersed in consumer products (e.g. crèmes) are underway.

Main Features (Equipment Capabilities):

Size Range Capability

The practical measurement range in any application depends upon the density of the particles to be measured. With very dense particles (for example, 6 times the density of water) the maximum and minimum sizes are smaller (/10 microns to under 0.005 micron). With low density particles (for example, from 0.85 g/cc to 1.10 g/cc) the maximum and minimum sizes are larger (/75 microns to /0.02 micron). The practical dynamic range (ratio of largest to smallest sizes) in a single analysis is about 70 using fixed centrifuge speed, and up to 1000 using ramping of speed during the analysis. For fixed speed, if the largest particle to be measured is 3 microns, then particles as small as 0.02 micron can normally be measured in a single analysis of less than 1 hour. For ramped speed, if the largest particle

to be measured is 10 microns, then particles less than 0.02 micron could normally be measured in a single run.

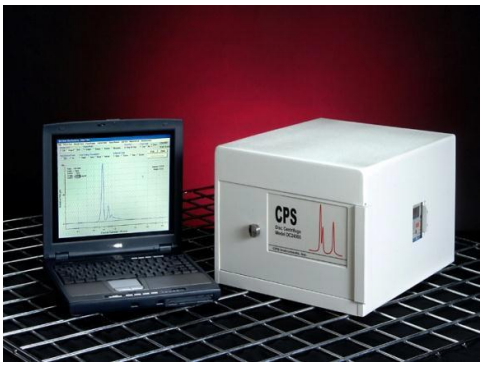
Analysis Time

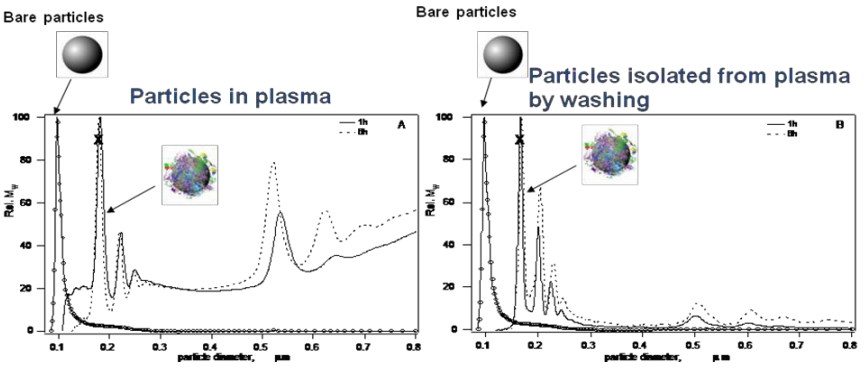
Analysis time will depend upon the range of sizes that is being analyzed and the density of the particles being measured. For the majority of applications, analysis times are in the range of 3 to 15 minutes fper sample. For extremely fine particles (0.005 to 0.06 micron), the analysis time can reach 30 minutes or more. CPS has developed techniques to allow analysis of most types of very small particles in a reasonable time. We can measure the size distribution of one or more of your specific samples to determine the exact analysis time prior to your application, in order to have a more accurate estimate of the visit duration requirements.

Issues to consider when designing TA experiments:

- Density of your particles – the lower limit of separation is dependent on the particle density;
- Concentration range for use in the measurements;
- When measuring nanomaterials size and size distribution in complex media, control samples in buffer or simple solutions should also be performed.

Typical Samples & Images:





DCS equipment, and example of determination of the effective size of nanoparticles in the absence (bare) and presence (corona) of human plasma. The corona is very similar *in situ* and following washing to remove the loosely bound proteins.

Key references:

- Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Bombelli FB, Dawson KA. Physical-chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. J Am Chem Soc. 2011, 133,2525-2534.
- Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the cell "sees" in bionanoscience. J Am Chem Soc. 2010, 132, 5761-5768.

Any further Information:

Although not specific as a UCD installation, proposals using DSC to characterise nanomaterial dispersions in situ in complex fluids can also perform measurements using dynamic light scattering (DLS) and NanoSight for comparison

of the sizes and size distributions by these methods.

If required, access to human plasma acquired using from the Dublin Blood Bank can also be provided. This should be indicated in the application form, along with the volume requested (typically 15 mls are sufficient for protein corona studies). If Users are bringing their own human biological samples, full ethical approval must be in place for this, and UCD requires copies in advance of any samples being brought to UCD.

The DCS installation can be used in conjunction with the EM installation for characterisation of nanomaterials in complex media. An example of such a comparison is shown in Walczyk et al., JACS, 2010, 132, 5761-5768.