Introduction
Nanotechnology refers to a field of applied science and technology that involves working with materials and devices on the nanoscale level, generally 100 nanometers (nm) or smaller. Virtually all industries and research areas are involved in the nanotechnology field including materials and chemicals, defense and security, tools and equipment, electronics and computers, and healthcare and biotechnology industries. Although many nanotechnology applications are industrial in nature, the combination of nanotechnology and biomedical sciences can lead to novel therapies such as targeted drug delivery, gene therapy, tissue engineering, targeting tumor destruction as well as diagnostics (new biomedical devices and biomarkers). Therefore, this research area is garnering more attention in recent years. The following application brief describes multiple methods for the separation of nanoparticles using Thermo Scientific Sorvall micro-ultracentrifuges and rotors.

Protocol 1: Procedure
Purification of single-walled carbon nanotubes by means of an ultracentrifuge.

The 1996 Nobel prize in Chemistry was awarded to Dr. Richard Errett Smalley for the finding of fullerene. The report by Dr. Richard Errett Smalley and his group members wrote in Science in July 2002 opened a new method of centrifugal separation and purification of carbon nanotubes (CNT).

By using heavy water as a suspension liquid, multi-bundle CNTs with a density higher than heavy water were precipitated and single-bundle CNTs that were less dense than heavy water remained in supernatant. The following report of sucrose density-gradient centrifugation considers the possibility of finer separation according to difference in chirality.

Centrifuge Conditions: Instrument
All centrifuge steps were performed using the Thermo Scientific MX 150 floor model micro-ultracentrifuge with the SS2-ST swinging-bucket rotor and 5mL polyallomer (PA) thin-walled tubes.

Separation Procedures

- **Speed:** 30,000 rpm
- **Maximum RCF:** 92,000 xg
- **Time:** 1 hour, 3 hours, 17 hours, and 34 hours
- **Temperature:** 20°C
- **Density gradient solution:** About 4.5 mL of 10 to 40% (w/w) sucrose continuous density gradient solution (heavy water used)
- **Sample:** About 0.5 mL of heavy-water suspension liquid containing sodium dodecyl sulfate (1% of single-walled carbon nanotubes)

Conclusion:
As the run time increases, the solution color becomes thinner due to dispersion and sedimentation of the black CNT particles. It suggests that CNT particles are separated according to size (sedimentation coefficient) or density. We consider that it is based on the difference in chirality.

There are many kinds of density gradient solutions other than sucrose. However, this kind of CNT sample generally contains relatively high-density surface-active agents such as sodium dodecyl sulfate (SDS). Therefore, non-ionic (non-electrolyte) density gradient solutions are preferable to ionic density gradient solutions.

References:
PROTOCOL 2: 
Procedure
Separation of ink using Thermo Scientific MTX 150 benchtop or MX Series floor model micro-ultracentrifuges and S140-AT fixed-angle rotor

With recent technological advances, the pigments contained in ink have become increasingly microparticulated—as small as a few nanometers in diameter. A normal centrifuge with a relative centrifugal force (RCF) of several tens of thousands of xg cannot sediment such nanometer size particles sufficiently.

Using the S140-AT rotor with a maximal g-force of 1,048,684 xg, we show that 6-10nm blank ink particles can be sedimented within 3.5 hours by using this rotor under the assumed conditions (particle density: 1.05 g/mL, particle configuration: perfect sphere, separation in distilled water). Furthermore, our calculations indicate that these particles can also be sufficiently separated in under two hours.

Centrifuge Conditions: 
Instrument
All centrifuge steps were performed using the Thermo Scientific MX 150 floor model micro-ultracentrifuge and the S140-AT fixed-angle rotor (2 mL x 10 tubes) with 2 mL polyallomer (PA) Re-seal tubes.

Separation Procedures
Speed: 140,000 rpm
Maximum RCF : 1,048,684 xg
Time: 3.5 hours
Temperature: 20°C
Sample: Black ink no.1 and 2

Result:

Conclusion:
The sizes of the particles sedimented in the above experiment are assumed to be between 6 and 10 nm for both the black in No.1 and No. 2. It shows effectiveness of the ultracentrifuge in separation sedimentation and concentration of nanometer-size particles.

Although the sample amount was low because the 2 mL tubes were used in the micro-ultracentrifuge this time, larger volumes (up to 138 mL of sample) can be separated in the larger volume floor model Sorvall WX Ultra centrifuge using the below suggested conditions.

Centrifuge Conditions: 
Instruments: Sorvall WX Ultra centrifuge and T-1270 fixed-angle rotor
Tube: 11.5 Polyallomer Ultracrimp tube
Speed: 70,000 rpm
Maximum RCF: 448,811 xg
Time: 24 hours
**PROTOCOL 3:**

**Procedure**

Separation of Mixed Nano-latex particles using the Sorvall MX 150 Floor Model micro-ultracentrifuge and the S52-ST swinging bucket rotor.

Nano-particles have been receiving particular attention owing to the breakthrough in nano-technology in recent years. An ultracentrifuge that is operable at hundreds of thousands of xg or higher RCF is helpful in separation and purification of nano-particles. The density gradient centrifugation is one of the ultracentrifugal methods. This method is further divided into the sedimentation velocity method and the sedimentation equilibrium method. Particles are separated by size according to the particle settling velocity, while, particles separated by the sedimentation equilibrium method are separated according to the differences in particle density. The following protocol is a report on the separation of 29 nm and 100 nm latex particles by the sedimentation equilibrium method.

**Centrifuge Conditions:**

**Instrument**

All centrifuge steps were performed using the Thermo Scientific MX 150 floor model micro-ultracentrifuge and the S52-ST swing-rotor with 5 mL polyallomer thin-walled tubes.

**Separation Procedures**

- **Speed:** 40,000 rpm
- **Maximum RCF:** 163,000 xg
- **Time:** 24 hours
- **Temperature:** 20°C
- **ACCEL/DECEL mode:** “8”/“8”

**Sample**

1. 0.1 mL of 29 nm polystyrene latex particles (Density 1.115 g/cm³)
2. 0.1 mL of 100 nm polystyrene latex particles (Density 1.060 g/cm³)
3. 0.2 mL of mixed particles (0.1 mL of 29 nm particles and 0.1 mL of 100 nm particles)

**Amount of Sample:** 2.7 mL

Density gradient solution: 4.3 mL of 5-30% sucrose solution

**Result:**

The centrifugation of the mixture of 29 nm particles (density 1.115 g/cm³) at 163,000 xg for 24 hours resulted in the formation of two bands. The band near the bottom consisted of 29 nm particles and the band near the top consisted of 100 nm particles. The result revealed the mixed latex particles were separated according to the difference in density, not the difference in particle size. The result also shows that an ultracentrifuge can separate nano-sized particles by the density-gradient equilibrium centrifugation method. It is a useful technique for separation of carbon nanoparticles or separation of fine pigments in ink or toner.

**Conclusion:**

The centrifugation of the mixture of 29 nm particles (density 1.115 g/cm³) at 163,000 xg for 24 hours resulted in the formation of two bands. The band near the bottom consisted of 29 nm particles and the band near the top consisted of 100 nm particles. The result revealed the mixed latex particles were separated according to the difference in density, not the difference in particle size. The result also shows that an ultracentrifuge can separate nano-sized particles by the density-gradient equilibrium centrifugation method. It is a useful technique for separation of carbon nanoparticles or separation of fine pigments in ink or toner.
Protocol 4: Procedure
Separation of metal nano-colloids using the Sorvall MTX 150 micro-ultracentrifuge and the S110-AT fixed-angle rotor

Metal nano-colloids are formed by dispersing metal particles 10 nm or smaller in surfactants or nonpolar solvents. These nano-colloids are used in making conductive inks, conductive coating materials and catalysts, and also in making biosensors. Pt, Pd, Fe, Co, Ni, Cu, Au, Ag, Ge, Sn and In are well known as single nanoparticles.

The following report describes a protocol for the separation and concentration of Ge particle samples having the lowest particle density among these single nanoparticles by using a micro-ultracentrifuge.

Centrifuge Conditions:
Instrument
All centrifuge steps were performed using the Thermo Scientific MTX 150 benchtop model micro-ultracentrifuge and the S110-AT fixed-angle rotor (5 mL x 8 tubes) with 5 mL polyallomer Re-seal tubes.

Separation Procedures
Speed: 100,000 rpm
Maximum RCF: 604,000 xg
Time: 7 hours
Temperature: 20°C
Sample: Ge particles (5 nm or smaller in particle size and 5.3 g/cm³ in particle density, made by K.K. Shinko Kagaku Kogyosho)
Sample volume: 5.1 mL

Result:
Before centrifugation
Precipitated Ge particles

After centrifugation
Supernatant
Precipitated Ge microparticles

Conclusion:
As shown in the experiment results, Ge particles 5 nm or smaller could be precipitated by centrifugation at about 600,000 xg for seven hours. For this reason, we considered that other metal particles will be also precipitated and concentrating in the same means. This experiment suggests that ultracentrifuge technology could be effectively used in the separation, purification and concentration of metal nano-colloids.

The Ge particles used in this experiment were provided by K.K. Shinko Kagaku Kogyosho.