

Ultracentrifugation

In-class Questions

You are likely familiar with a device called a centrifuge where a sample is put into a suitable holder and spun. The spinning creates a centrifugal force that is greater than gravity, with the result that the centrifugal force causes particles in the sample holder to settle out.

Q1. What are the variables that influence the settling of a particle in a centrifuge?

Q2. What does the name ultracentrifuge imply?

Q3. Suppose you wanted to separate molecules or particles using an ultracentrifugation procedure. What variables could you alter that would allow you to complete the separation?

Q4. How could you use the speed of the centrifuge or time that the sample has been allowed to spin to perform a separation of several different components of the mixture?

A second variable is the density of the solvent in the centrifuge tube.

Q5. Can you think of a procedure based on solvent density that could be used to separate several different components of a mixture in an ultracentrifuge?

Q6. Can you think of a procedure for generating a density gradient in a centrifuge tube?

Ultracentrifugation

You are likely familiar with a device called a centrifuge where a sample is put into a suitable holder and spun. The spinning creates a centrifugal force that is greater than gravity, with the result that the centrifugal force causes particles in the sample holder to settle out.

Q1. What are the variables that influence the settling of a particle in a centrifuge?

There are a number of variables that influence the settling of a particle. Obvious ones are the speed or frequency at which the sample rotates and the mass, density and size of the particle. Another one that may not be as obvious is the density or viscosity of the solvent that the sample is in.

Q2. What does the name ultracentrifuge imply?

The name ultracentrifuge implies that samples are rotated at a very high speed. Ultracentrifuges operate at speeds of more than about 20,000 revolutions per minute. The ultrahigh speed of a centrifuge allows you to settle out much smaller particles. In fact, the speed of an ultracentrifuge is high enough that large molecules such as proteins, nucleic acids, and other polymers settle differently based on their molecular weights. Ultracentrifugation is often used to isolate cells, subcellular organelles, and macromolecules.

Q3. Suppose you wanted to separate molecules or particles using an ultracentrifugation procedure. What variables could you alter that would allow you to complete the separation?

One variable is the speed of the centrifuge and the time the sample has been allowed to spin in the centrifuge.

Q4. How could you use the speed of the centrifuge or time that the sample has been allowed to spin to perform a separation of several different components of the mixture?

You could use a process known as **differential centrifugation**. Larger particles will settle faster and at slower speeds than smaller particles. The materials that settle to the bottom of the centrifuge tube are known as the pellet. It is possible to pellet a subset of material in the tube, remove the supernatant and add it to a second tube, up the spin rate to create a second pellet, and so on. The pellets obtained from a differential centrifugation are often not pure enough for follow-up studies and must be subjected to additional purification.

A second variable is the density of the solvent in the centrifuge tube.

Q5. Can you think of a procedure based on solvent density that could be used to separate several different components of a mixture in an ultracentrifuge?

You could use a process known as **density gradient centrifugation**. This is the preferred mechanism to purify compounds using ultracentrifugation. The heaviest or densest layer is placed at the bottom of the tube while the lightest layer is at the top. The sample is then placed on top of the gradient and the sample spun in the centrifuge. The most common materials used to generate density gradients are sucrose or cesium iodide.

Q6. Can you think of a procedure for generating a density gradient in a centrifuge tube?

One procedure would be quite similar to the process used to perform a gradient elution separation in liquid chromatography. This procedure would involve preparing solutions at the two extremes of the gradient you want to create in the tube. You would start with the heavier solution, and then using two pumps, systematically reduce the amount of the heavier solution while increasing the amount of lighter solution. The two solutions coming from the pumps must be mixed for homogeneity and then carefully added to the centrifuge tube such that the layers of different densities do not mix with each other. Density gradient centrifugation can be used to separate substances on the basis of size or mass (a **rate zonal separation**) or density (an **isopycnic separation**).

A **rate zonal separation** has certain aspects similar to a differential centrifugation. The sample is introduced at the top of the gradient and allowed to spin for a certain period of time during which the particles migrate at different rates toward the bottom of the tube. There are several important criteria that are needed for a successful rate zonal separation.

- The sample solution must have a density that is lower than the lowest density portion of the gradient.
- The density of the particles being separated must be greater than the highest density portion of the gradient.
- The density gradient must have a sufficiently long pathlength so that the separation can occur.
- The separation cannot be run for too long a time, otherwise all of the particles can form a single pellet at the bottom of the tube.

In an **isopycnic separation**, the particles will sink into the gradient to the point where the density of the gradient equals the density of the particle (there isopycnic point). Once there, the particles will remain suspended at that point in the gradient irrespective of the length of time that the sample is kept in the centrifuge. There are several criteria that are needed for a successful isopycnic separation.

- The density of the particles must fall between the density limits of the gradient.
- The time must be long enough so that each particle has reached its isopycnic point.