Developing the Ultracentrifuge

Christopher S. W. Koehler

Throughout the 20th century, a succession of researchers contributed to the gravity of the situation.

The history of the development of the ultracentrifuge is a story of the intersection of basic physics and practical chemistry with results that helped create the field of molecular biology. The ultracentrifuge can generate forces thousands or millions of times stronger than the force of gravity. The development of this class of devices permitted the fractionation of subcellular bodies previously visible only through an electron microscope. This in turn facilitated the analysis of their enzymatic and molecular constituents, providing information about structure and function.

On the Centrifuge

The simplest centrifuge is a rotor mounted on a shaft and powered by an external device. Common examples include an electric fan or the wheels of a car. Other examples include windmills, in which moving air drives a shaft-mounted rotor, or waterwheels, in which water does the same thing.

But these simple centrifuges, and small-scale models used in the laboratory, have some serious drawbacks that stem from the basic physics of a shaft-mounted rotor. Spinning shaft-mounted rotors at speeds greater than a few hundred revolutions per second (rps) cause a problem much like wheel imbalance in a car. But where a car's wheels rotate at perhaps only 10 rps even at freeway speeds, the rotor of a centrifuge can turn at several hundred (or more) rps. At those rates, the inability to make the inertial axis of the rotor match exactly the axis of its motive shaft becomes problematic, and the rotor shakes uncontrollably.

Carl de Laval, a Swedish engineer, overcame some of these limitations in 1883, when he introduced a turbine that featured a steam-powered rotor on a flexible shaft. This shaft was able to shift under the force of the imbalance and could turn at several hundred rps.

In addition to the mismatch between the axial rotation of the rotor and the shaft, there were other drawbacks and limitations of this technology that only became apparent with the discovery of the large macromolecules. The slower speeds necessary to keep the centrifuge from shaking itself apart were too slow to separate the macromolecules that increasingly drew the attention of biologists, chemists, and physicists after Friedrich Miescher isolated DNA.

Theodor Svedberg at the Gustav Werner Institute, 1953

PHOTO COURTESY OF THE SVEDBERG LABORATORY, UPPSALA UNIVERSITY, UPPSALA, SWEDEN

But spinning the centrifuges at speeds high enough to separate macromolecules would cause the centrifuge to break apart under the influence of the forces it generated, sometimes forcefully.

Svedberg and the Ultracentrifuge

In the 1920s, Theodor H. E. Svedberg at Upsalla University became interested in the problems of centrifugation through chemistry. Svedberg was a colloid chemist. His doctoral dissertation described a new method of producing colloid particles and provided convincing proof of a theory by Albert Einstein and Marian Smoluchowski concerning Brownian motion, which provided proof of the existence of molecules. With several collaborators, Svedberg went on to investigate the properties of colloids, including diffusion, light absorption, and sedimentation.

It was through his studies of colloid sedimentation that Svedberg came to invent the first of the ultracentrifuges, centrifuges that operated at thousands of rps and were powerful enough to separate even macromolecules.

Svedberg developed his ultracentrifuges in the 1920s in the hopes that they might yield the answer to what was at the time thought to be the key to colloid solutions: the distribution of particle size. In the ultracentrifuge, large molecules such as proteins and carbohydrates were spun fast enough to subject them to thousands of times the force of gravity, ultimately up to about 10^6 g. This was in 1925–1926.

Svedberg and Alf Lysholm at Uppsala later constructed a centrifuge with a maximum speed of 42,000 rpm. Svedberg's initial high-speed centrifuges were small, with the rotor mounted on a nonflexible shaft. Their speeds topped out around 1000 rps in a normal atmosphere. However, when the rotor was housed in a low-pressure hydrogen atmosphere, he was able to subject colloid and other samples to forces up to a million times that of gravity.

The protein–colloid solutions Svedberg wished to study were enclosed in plane parallel rock crystal cells. Each such setup was covered with a layer of vacuum oil and placed in a rotor mounted atop a vertical shaft, which was equipped with a thermocouple-controlled cooling system. The rotor was enclosed in a hydrogen atmosphere because hydrogen provides much less friction and equalizes temperature differences. The housing had two windows for

a beam of UV light to be shone through the sample during rotation. Conditions were detected with a UV camera.

Svedberg's results convinced him that proteins were formed by aggregates of a single very large unit. He thought, wrongly as it turned out, that all proteins were multiples of this basic unit, which came to be called "the Svedberg"—which had a molecular weight of 17,500 Da. Using his ultracentrifuge techniques, Svedberg calculated the molecular weights of hemoglobin and casein. By the end of the 1920s, his research, which had earned him a Nobel Prize in chemistry in 1926, headed in the direction of biomolecular surveys and molecular measurements of phylogenetic relationships.

Beams and the Ultracentrifuge

Jessie Beams, an American physicist associated with the University of Virginia (UVA), was interested in high-speed centrifugation as well. The centrifuge design Beams was familiar with was startlingly different from that developed by Svedberg. Two Belgian scientists, E. Henriot and E. Huguenard, had developed a shaftless centrifuge. Its rotor was driven by air and suspended in space by a jet of air. Gone was the shaft and so gone was the problem of mismatch between the axial rotations. Shaftless rotors as small as an inch in diameter were free to spin up to 4000 rps. The primary drag on speed was atmospheric friction.

As a young researcher at UVA, Beams contemplated the new applications for the technology that could result if the drag on the rotational speed could be eliminated: eliminate or reduce the drag and rotational speeds of a million or more rps might be possible. As a result, he concentrated his research and design efforts on drag factors.

Beams realized that his rotors would have to be enclosed in a high vacuum if his ultracentrifuge was to overcome the limitations of previous designs, like those pioneered by Svedberg. His first designs featured rotors suspended in a vacuum from a flexible shaft. The shaft passed through an oil seal to attach to an air-driven turbine. Like the early system of de Laval, this flexible shaft allowed the rotor to spin about its own inertial axis. This design was obviously far from frictionless, but it avoided the problem of frictional sample heating and it permitted rotors up to a foot in diameter to spin at thousands of rps.

Although useful—Beams in 1961 described this type of centrifuge as the "workhorse" of molecular sedimentation experiments—this was not the ultimate ultracentrifuge Beams sought and knew could be designed. Rather, he was looking for a centrifuge in which the tensile strength of the rotor alone limited the rate of spin. To reach this, the vacuum would have to be strong and there could be no supporting shaft.

In the mid-1930s, Beams experimented with magnetic fields to support the rotor. The field generated by an external electromagnet could lift a rotor made of ferromagnetic material. Such a rotor would spin freely and seek to rotate unimpeded about its own inertial axis.

Beams hung the electromagnet by a flexible wire so that the ferromagnetic rotor could pull the axis of the electromagnetic field in line with its own axis of rotation, stabilizing the spin axis at very high speeds.

Another problem remained. Such a rotor could range up and down that vertical axis if the balance between the magnetic field and gravitational pull was not maintained. Beams' solution was ingenious. He flashed a beam of light across the rotor to a photoelectric cell. If the rotor deviated, the light hitting the photoelectric cell would change in such a way that it produced a corresponding current in the electromagnet. This then corrected the rotor position.

With the rotor in its evacuated chamber stabilized by external fields, the ultracentrifuge could spin rotors of less than a thousandth of an inch to more than a foot, and of weights ranging from a billionth of a pound to more than a hundred pounds. The rotor could spin to speeds of more than a million rps, speeds at which the rotor could easily explode under centrifugal forces of more than a billion times the force of gravity.

Applications and Improvements

A host of workers at many institutions used these and newer types of ultracentrifuges to make significant advances. Rockefeller Foundation money helped to fund Ralph Wyckoff's development of an air-driven ultracentrifuge, a simpler and cheaper device than Svedberg's original design, which was important to the virus studies of W. M. Stanley, for example. Beams ultimately used the field-supported model to separate atomic isotopes, contributing to the development of the atomic bomb. He also developed ways to establish the strength of materials by driving the rotors to explosive speeds.

Others continued to elaborate on the basic design. Edward Pickels, one of Beams' students, developed an ultracentrifuge driven by electricity and helped develop one of the earliest preparative ultracentrifuges marketed by Spinco (Specialized Instruments Corp.) which was acquired by Beckman in 1955.

The Beckman Model E ultracentrifuge developed after World War II became the workhorse for analytical ultracentrifugation; later, in the 1960s, Yoichiro Ito developed a centrifuge based on a coil, the coil planet centrifuge, that led to the development of countercurrent chromatography in the early 1970s. Throughout the latter half of the 20th century, preparative and analytical ultracentrifugation took their places among the key technologies of modern chemistry, especially the realm of molecular biology.

Suggested Reading

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Christopher S. W. Koehler *holds a Ph.D. in the history of science. He writes and teaches in northern California. Send your comments or questions about this article to tcaw@acs.org or to the Editorial Office address on page 3.* ◆