Continuous Flow Ultracentrifuge System for Production of Infection Prevention Vaccines

Masataka Morita
Masaharu Aizawa
Hiroatsu Toi
Eiichi Fukuhara
Kazuhisa Hashimoto

OVERVIEW: Given the concerns about the potential for an epidemic of avian influenza similar or even worse than the 2009 H1N1 flu pandemic, it is hoped that vaccines will be able to play an even greater role in fighting these infectious diseases. Centrifuges that operate at very high speed are used in the separation and purification of pathogenic viruses, antigens, and other agents used in the production of vaccines and other pharmaceuticals and Hitachi Koki Co., Ltd. has for more than 20 years been producing continuous flow ultracentrifuges that it supplies to major pharmaceutical and vaccine manufacturers in Japan and elsewhere. The demand from pharmaceutical and vaccine manufacturers is for production machinery that can improve quality levels in the manufacture of pharmaceuticals and increase production efficiency. By supplying such equipment, Hitachi Koki is helping create a comfortable society and a healthy population.

INTRODUCTION

CENTRIFUGES have a long history, having been used to separate cream from milk since the latter part of the 19th century. They went on to be used in applications such as the separation of animal or vegetable oils and in brewing, but these centrifuges did not require a very high level of centrifugal force.

Subsequently, progress in the field of biochemistry led to demand for centrifuges capable of higher levels of centrifugal force, including high-speed centrifuges produced in the 1930s that were used for separating cell components such as cell membranes and mitochondria. This was followed by growing demand for virus separation for use in virus research and led to the development of ultracentrifuges with top speeds of 40,000 min^{-1} and centrifugal accelerations of 100,000 G (100,000 times the gravitational acceleration of earth) or more in the 1950s. Subsequently, a large continuous flow ultracentrifuge with a top speed of 35,000 min^{-1} driven by an air turbine was developed in the USA in 1968.

Fig. 1—Hitachi CS150NX Desktop Ultracentrifuge. Featuring a top speed of 150,000 min^{-1}, centrifugal acceleration of 1,050,000 G, and separation capacity of 180 ml, the CS150NX is used for research in fields such as medicine and biochemistry.

As part of a government-sponsored project to develop a centrifuge for use in research in which Central Research Laboratory, Hitachi, Ltd. was also involved, Hitachi Koki undertook the production of a prototype centrifuge in 1952. Hitachi Koki then went on to turn the centrifuge into a commercial product and in 1988 developed a low-noise continuous flow ultracentrifuge with a top speed of 40,000 min^{-1} driven by a high-frequency motor. Hitachi Koki has now become one of the leading general centrifuge producers in the world with an extensive product range that extends from a research ultracentrifuge with a top speed of 150,000 min^{-1} and centrifugal acceleration of 1,050,000 G (see Fig. 1) through to continuous flow ultracentrifuges and desktop centrifuges (4,000 min^{-1}, 2,000 G) used in hospitals for preprocessing blood, urine, and other samples.

This article gives an overview of continuous flow ultracentrifuges and describes the technical points and other features of the Hitachi CC40NX continuous flow ultracentrifuge that went on sale in October 2009.

OVERVIEW OF CONTINUOUS FLOW ULTRACENTRIFUGES

Structure of Continuous Flow Ultracentrifuges

Continuous flow ultracentrifuges are used in the production of vaccines, primarily those that protect against infectious viral diseases (such as influenza and

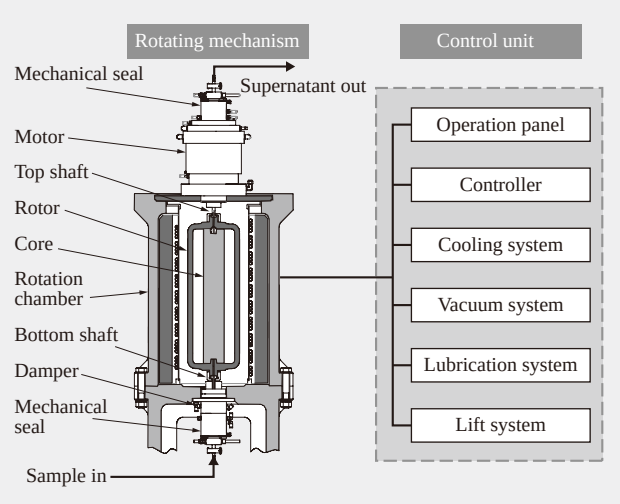

Fig. 2—Structure of Rotating Mechanism and Control Unit. The centrifuge is driven by a high-frequency motor. The sample is introduced via the bottom mechanical seal, separation takes place inside the rotor, and then the supernatant (leftover material) is discharged from the top mechanical seal.

Japanese encephalitis). They are used to separate and extract the desired material from a culture medium in which pathogenic viruses or other useful substances have been cultured in large quantities. Continuous flow ultracentrifuges are an important part of vaccine production because these pathogenic viruses or other useful substances exist in the form of very small particles (18 to 300 nm)[(1)] and centrifuges provide a particularly effective way of separating, purifying, and concentrating them.

A continuous flow ultracentrifuge consists of a rotating mechanism and a control unit and works by rotating a cylindrical rotor at very high speed and continuously introducing the sample via the top and bottom hollow shafts that support the rotor so that the desired material can separate out inside the rotor and the supernatant (leftover material) can be continuously discharged (see Fig. 2).

The rotating mechanism has a cylindrical rotor in the center that is linked to drive shafts at the top and bottom ends. The shafts are hollow to allow the sample to pass through and are supported by bearings at the top and bottom. The top shaft connects to the high-frequency motor. The ends of the top and bottom shafts also have mechanical seals where the sample passes between the static and rotating parts.

Because the rotor is able to operate at speeds up to 40,000 min^{-1} giving it a very high maximum acceleration of 118,000 G, it is made of high-strength titanium alloy with high specific strength and excellent corrosion resistance.

The control unit includes an operation panel for setting the operating parameters and displaying status information, a controller that performs electrical control of the centrifuge including the motor and inverter, two cooling systems that cool the rotating rotor and the motor as well as mechanical seals, a vacuum system that creates a vacuum inside the rotor chamber to prevent heat generation by the rapidly rotating rotor, and a lift system for raising the rotor in the rotating mechanism so that it can be inserted and removed.

Continuous Flow Ultracentrifuge Applications and Procedure for Use

Applications for continuous flow ultracentrifuges include separation of viruses such as those for influenza or Japanese encephalitis which are used to produce vaccines and separation of compounds used in diagnostic reagents for diseases including adult T-cell leukemia and AIDS (acquired immune deficiency syndrome) (see Table 1). The production of influenza vaccines represents a typical example (see Fig. 3).

Currently, the production of influenza vaccines in Japan involves introducing a viral strain derived from pathogenic viruses into fertilized chicken eggs under controlled conditions in which they are then incubated.

TABLE 1. Example Continuous Flow Ultracentrifuge Applications
The centrifuge is used in the production of vaccines and various diagnostic reagents.

Application	Purpose	Separation method, speed
Influenza vaccine	Purification of pathogenic virus	Continuous, 35,000 min^{-1}
Japanese encephalitis vaccine	Purification of pathogenic virus	Continuous, 35,000 min^{-1}
Hepatitis B vaccine	Purification of useful material	Discontinuous flow centrifugation, 34,000 min^{-1}
Rabies vaccine	Purification of pathogenic virus	Continuous, 35,000 min^{-1}

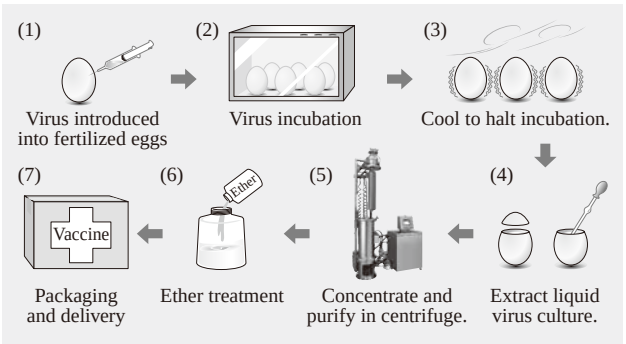

Fig. 3—Example of Influenza Vaccine Production. In Japan, influenza vaccine is produced from fertilized eggs.

After incubation, the viral particles are extracted using sucrose density-gradient centrifugation[2] and treated with ether.

The ultracentrifugation methods used in this process are "continuous flow centrifugation" which forms a density gradient in the fluid inside the rotor and continuously injects sample so that the desired material is isolated within a density gradient band and "discontinuous flow centrifugation" which also uses a density gradient to isolate the desired material but uses a premixed sample (sample mixed in density gradient fluid) rather than a continuous flow. The choice of separation method is determined by factors such as the particle size of the material to be separated, the density with which it is suspended in the solution, and how its properties differ from the unwanted material. A feature of the continuous flow centrifugation method widely used to separate the material required for vaccines is that it can process 50 to 100 L of sample at a time despite the rotor capacity being only 3 to 8 L.

Separation of influenza viruses is performed using continuous flow centrifugation of a fluid with a sucrose density gradient (see Fig. 4). The procedure is summarized below.

Step 1: With the rotor stationary, volumes of buffer and 60% (w/w) sucrose solution respectively equivalent to 50% of the rotor capacity are injected into the rotor from the bottom shaft. The buffer is a solution in which the concentration of hydrogen ions has been adjusted to prevent loss of activity by the biological sample.

Step 2: The rotor is accelerated to 4,000 min^{-1}. This rotation causes the two layers of solution injected in step 1 to form a vertical arrangement with the 60% sucrose solution concentrated toward the inner wall of the rotor and the buffer toward the center (inside the sucrose solution). Diffusion in the boundary between these two regions forms a density gradient in the form of band in which the sucrose concentration goes from 0 to 60%.

Step 3: With the centrifuge operating at a fast specified speed (typically 35,000 min^{-1}), sample is injected at a constant rate. This injection rate is the rate at which the viral material is isolated in the rotor (10 to 20 L/h). The viral particles separate out inside the rotor and aggregate at the density position equivalent to the virus's own suspension density (corresponding to a concentration of about 42%).

Step 4: The centrifuge continues to operate at the specified speed after sample injection finishes to allow the particles inside the rotor to fully separate out.

Step 5: The speed is reduced to 4,000 min^{-1} and then the centrifuge is slowly brought to a halt so as not to disrupt the region of liquid where the density gradient has formed.

Step 6: After rotation halts, the liquid is extracted from the rotor and the target material recovered.

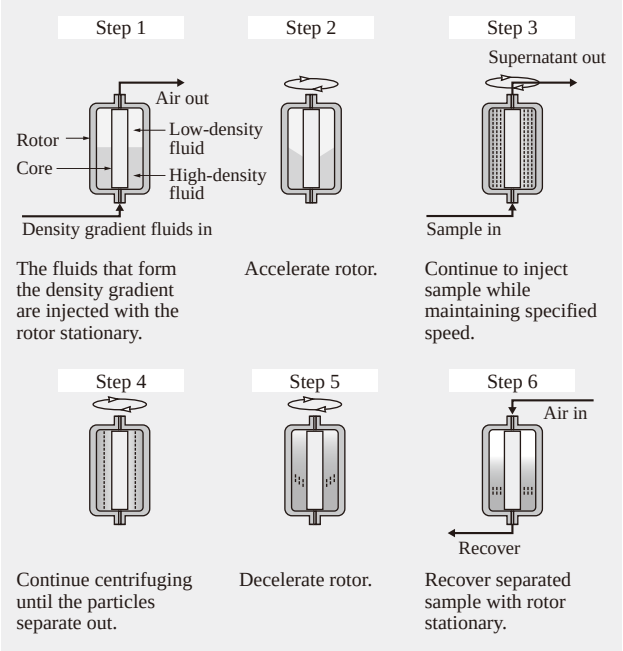

Fig. 4—Operating Procedure for Continuous Flow Centrifugation. Continuous flow centrifugation forms a density gradient in the fluid inside the rotor. This causes the target material to separate out in the density gradient band from which it can be recovered.

CONTINUOUS FLOW ULTRACENTRIFUGE SPECIFICATIONS AND CUSTOMER REQUIREMENTS

Table 2 lists the main specifications of a continuous flow ultracentrifuge.

The demand from pharmaceutical and vaccine manufacturers is for production machinery that can improve the quality of pharmaceuticals and increase production efficiency. The key points are as follows.

TABLE 2. Main Specifications of Continuous Flow Ultracentrifuge
The performance of the ultracentrifuge allows it to be used to separate microparticles.

Maximum speed	40,000 min^{-1}
Maximum centrifugal acceleration	118,000 G
Maximum rotor capacity	8 L
Drive mechanism	Direct drive using high-frequency motor
Dimensions	1,750 (W) × 1,150 (D) × 2,950 (H) (mm)
Weight	900kg

(1) Improved sterility of separation process

Hygiene management in pharmaceutical manufacturing requires the rigorous maintenance of a clean environment free of microorganisms, toxins, and other contaminants. The areas covered by this hygiene management include the flow path through the centrifuge (the places where the centrifuge comes into contact with the sample). The sterilization methods used on past machines, however, were subject to limitations and involved only immersion sterilization in a reagent prior to spinning up. In the case of pharmaceutical production equipment, the requirement is to be able to use SIP (steam-in-place or sterilization-in-place)[*1] which provides a higher degree of sterilization.

(2) Electronic data management of pharmaceutical production records

Pharmaceutical manufacturing requires the collection and storage of detailed records including status information and other operational data from production equipment and in the past this has been done using paper records. There is a need to improve this process by using computer-based record collection and electronic approvals to achieve a higher standard of quality records and more reliable record-keeping.

(3) Flexible products for use with pharmaceutical production equipment

Because of the wide range of different pharmaceutical production equipment used at different sites, the compatibility between the centrifuge and other production equipment can have a major influence on things like productivity and manufacturing quality. Accordingly, it is necessary to supply products with configurations and specifications that suit a variety of different customer sites.

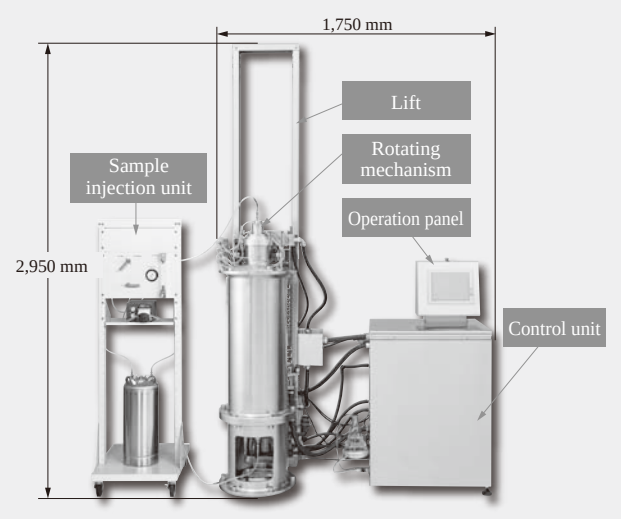

Fig. 5—Hitachi CC40NX Continuous Flow Ultracentrifuge. First released in October 2009, the ultracentrifuge has a maximum speed of 40,000 min^{-1} and numerous features including support for steam sterilization of the sample flow path and electronic recording of operating data.

Fig. 6—Titanium Rotor Core and Operation Panel. The lightweight titanium core is inserted into the rotor and provides a separation layer in the resulting gap. The operation panel provides a graphic display of operating status information.

CC40NX CONTINUOUS FLOW ULTRACENTRIFUGE FEATURES AND TECHNOLOGY DEVELOPMENT

SIP Compatibility

The CC40NX (see Fig. 5) was released in October 2009. To ensure that SIP could be used, its development process included: (1) Use of a titanium alloy core with a lightweight design for inserting into the rotor in place of a plastic core [see Fig. 6 (a)], (2) Use of heat-resistant materials in the sample flow path components, and (3) A control method for the centrifuge unit during steam sterilization. These developments meant that the CC40NX was the first continuous flow ultracentrifuge (over 100,000 G) able to be sterilized using high-pressure steam (121°C for 20 min) without taking it out of the pharmaceutical production line.

Electronic Recording of Operating Data and Color Touch Panel Screen

The centrifuge includes a built-in control computer that can automatically collect operational data during pharmaceutical production. The collected data complies with the quality management standards of the US Food and Drug Administration (FDA) as well as producing electronic records and electronic signatures that satisfy the US FDA 21 CFR (Code of Federal Regulations) Part 11 rules for food and drugs.

[*1] Steam-in-place or sterilization-in-place refers to sterilization processes that can be used without dismantling the equipment and that reduce the presence of microorganisms in the sample flow path to less than a stipulated level.

Other features include a login function that requires a user ID (identification) and password, recording of operating status information, Audit Trail function, and security functions.

The operation panel uses a color LCD (liquid crystal display) touch screen that is easy to operate. Its capabilities include being able to display both settings and operating status information at the same time, graphical display of operating status information, and a multilingual display function [see Fig. 6 (b)].

Flexible System Able to Adapt to Customer Needs

Because of the variety of pharmaceutical production equipment used at different sites, the centrifuge was designed to have the flexibility to adapt to each customer site.

(1) Improved suitability for automated lines

The centrifuge can incorporate hardware-level connectivity with automatic sample supply and extraction systems and communication capabilities that allow connection to customers' networks.

(2) Support for biohazards and cleanrooms

The centrifuge can satisfy a range of different requirements depending on the properties of the samples being processed. Examples include the ability to install an HEPA (high-efficiency particulate air) filter in the vacuum extraction line from the chamber, improved corrosion resistance to withstand the reagents used to perform room-wide disinfection in rooms where the centrifuge may be housed, and support for remote operation of a split configuration in which the rotating unit is located in a separate isolated room and the control unit in a standard room.

The extent to which the centrifuge gives off particles can also be reduced to facilitate its use in a cleanroom.

CONCLUSIONS

This article has given an overview of the continuous flow ultracentrifuges used to produce vaccines and other pharmaceuticals and described the technical points and other features of the Hitachi CC40NX continuous flow ultracentrifuge.

The methods used for influenza vaccine production are shifting toward use of cultured cells derived from animals such as monkeys or dogs in order to shorten the time taken to produce the vaccines and to avoid side effects caused by proteins that originated in eggs. Despite these changes, centrifuges remain an effective way of separating and purifying the required material and it seems likely that continuous flow ultracentrifuges will remain in use in the future.

Hitachi Koki Co., Ltd. intends to continue working toward the creation of a comfortable society and a healthy population by supplying products that meet customer needs.

REFERENCES

(1) "Experimental Virology (various editions)," Publication of the National Institute of Infectious Diseases, Maruzen Co., Ltd. (1982) in Japanese.

(2) D. Rickwood, "Preparative Centrifugation," IRL Press Ltd., Oxford (1992).

ABOUT THE AUTHORS

Masataka Morita

Joined Hitachi Koki Co., Ltd. in 1980, and now works at the Planning Department, Life-Science Instruments Division. He is currently engaged in the application of centrifuges and centrifugation. Mr. Morita is a member of The Japanese Society for Vaccinology and The Japanese Biochemical Society.

Masaharu Aizawa

Joined Hitachi Koki Co., Ltd. in 1968, and formerly worked at the Design Department, Life-Science Instruments Division.

Hiroatsu Toi

Joined Hitachi Koki Co., Ltd. in 1996, and now works at the Design Department, Life-Science Instruments Division. He is currently engaged in the development of continuous flow ultracentrifuges.

Eiichi Fukuhara

Joined Hitachi Koki Co., Ltd. in 2008, and now works at the Design Department, Life-Science Instruments Division. He is currently engaged in the development of continuous flow ultracentrifuges.

Kazuhisa Hashimoto

Joined Hitachi Koki Co., Ltd. in 1985, and now works at the Domestic Sales & Marketing Department, Life-Science Instruments Division. He is currently engaged in the planning and marketing of centrifuges.