AIP | Review of Scientific Instruments

Assembly and Operation of a Simple, Multipurpose Zonal Ultracentrifugation System

J. E. Paris

Citation: Review of Scientific Instruments **41**, 90 (1970); doi: 10.1063/1.1684288
View online: http://dx.doi.org/10.1063/1.1684288
View Table of Contents: http://scitation.aip.org/content/aip/journal/rsi/41/1?ver=pdfcov
Published by the AIP Publishing

Articles you may be interested in

Multipurpose headphone assembly
J. Acoust. Soc. Am. **76**, 1883 (1984); 10.1121/1.391556

Automatic Sequence and Photo Control System for Simultaneous Ultracentrifugation in the Analytical Ultracentrifuge
Rev. Sci. Instrum. **38**, 1463 (1967); 10.1063/1.1720562

A Reflecting Mirror Optical System for the Ultracentrifuge
Rev. Sci. Instrum. **12**, 32 (1941); 10.1063/1.1769775

Design and Operation of the Oil-Turbine Ultracentrifuge
Rev. Sci. Instrum. **9**, 346 (1938); 10.1063/1.1752364

Ultracentrifuge
J. Chem. Phys. **2**, 143 (1934); 10.1063/1.1749437

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://scitationnew.aip.org/termsconditions. Downloaded to IP: 35.8.11.3 On: Thu, 28 Jan 2016 11:41:14

ment the nozzle temperature was reduced slightly by pumping on the helium reservoir. A velocity spectrum derived from the time spectrum is shown in Fig. 7. A good fit to the experimental data, which has an FWHM of 53 m/sec, is achieved using $T_0 = 5.9$ K and $M = 10$ in Eq. (3). Corrections to the experimental width of the velocity spectrum for the width of the chopper slit and the length of the ion gauge detector are calculated to be of the order of 5% and are neglected.

A beam of polarized $^3He^+$ ions suitable for injection into a particle accelerator can be produced by passing the neutral beam through a hexapole magnet as shown in Fig. 1.[1] As the neutral atoms traverse the hexapole magnet, those in the nuclear spin substate $m_I = -\frac{1}{2}$ are focused towards the central axis, while those in the spin substate $m_I = +\frac{1}{2}$ are defocused and hence removed from the beam. A charged beam can then be produced by electron bombardment. With a hexapole magnet 50 cm long having an entrance diameter of 0.3 cm and an ionizer with ionization efficiency of 0.1% the present atomic beam source should produce a polarized $^3He^+$ beam with an intensity of the order of 10 nA.

This work shows that cryogenically cooled nozzle sources provide an excellent means of producing beams of very slow helium atoms with low velocities and small velocity spreads. Reducing the background gas pressure in the nozzle–skimmer region and better thermal contact of the nozzle to the liquid helium reservoir should allow the attainment of still higher beam intensities and lower velocities than presented here.

THE REVIEW OF SCIENTIFIC INSTRUMENTS VOLUME 41, NUMBER 1 JANUARY 1970

Assembly and Operation of a Simple, Multipurpose Zonal Ultracentrifugation System

J. E. PARIS*
Department of Radiology, University of Maryland Hospital, Baltimore, Maryland 21201
(Received 9 April 1969)

A description is given for the correct assembly and operation of the variety of components of a zonal ultracentrifugation system, useful for several kinds of zonal particle separations. Simple, reproducible means are provided for the storing, volume measurement, and output control of the gradient, sample, and overlay solutions. These fluids do not come in contact with lubricated parts. The liquid flux along the lines is free from pressure pulses or local areas of turbulence. The various solutions are kept cold without installation of the whole setup in a temperature controlled environment. The system of connections is designed to be smooth and compact, with a minimum of dead spaces. The type and size of the necessary tubing connectors, stopcocks, etc. was chosen as to preserve lamellar flux of gradient solutions and to insure permanent tightness. The proposed system can be easily disassembled for the purpose of cleaning and a large number of experiments did not affect its hermeticity. All parts in contact with solutions are autoclavable for sterilization.

ZONAL ultracentrifugation in sector shaped rotors is a technique recently developed for the mass separation of subcellular entities, viruses, or other particles on the basis of their differences in buoyant density or sedimentation ratio.[1–3] Low, intermediate, and high speed zonal rotors are already available for operation in the several centrifuges and ultracentrifuges adaptable to zonal work.[1] For the correct assembly and operation of the variety of components of a zonal centrifugation system, however, it is usually necessary to perform a time consuming series of trials in order to design a layout meeting the requirements of the planned particle separation.

The following prerequisites were taken in consideration during the planning of a zonal centrifugation system in our laboratory.

Simple, reproducible means should be provided for the storing, volume measurement, and output control of the gradient, sample, and overlay solution. These fluids must not come in contact with lubricated parts. The liquid flux along the lines should be free from pressure pulses or local areas of turbulence.

For work with biological samples, the various solutions must be kept cold. Unless strictly necessary, cumbersome installation of the whole setup in temperature controlled environment will not be considered.

The diameter of the required hose connections should be chosen as to insure a suitable flow rate of the generally viscous solutions employed, while being kept small enough to prevent back-mixing of gradients. The system of connections should be designed as smooth and compact as possible, with a minimum of dead spaces.

Quick change of solution reservoirs during operation should be done without replacement or reconnection of the hose system. The type and size of the necessary tubing

* Present address: Department of Biochemistry, Tufts University, Boston, Mass. 02111.

[1] N. G. Anderson, *et al.*, J. Nat. Cancer Inst. Monograph **21**, 1966.
[2] F. Leighton, B. Poole, H. Beaufay, P. Baudhuin, J. W. Coffey, S. Fowler, and C. de Duve, J. Cell. Biol. **37**, 482 (1968).
[3] H. Schuel and N. G. Anderson, J. Cell. Biol. **21**, 309 (1964).

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://scitationnew.aip.org/termsconditions. Downloaded to IP: 35.8.11.3 On: Thu, 28 Jan 2016 11:41:14

TABLE I. Description and source of components for multipurpose zonal ultracentrifugation system.

Component	Supplier	Address	Catalog No.
Masterflex tubing pump, with solid state speed controller	Cole Parmer	Chicago, Ill.	7020 V 15
Tygon tubing for Masterflex pump i.d. 4.76 mm, o.d. 9.5 mm	Cole Parmer	Chicago, Ill.	6408
Tygon tubing for all other connections i.d. 3.17 mm, o.d. 6.35 mm	A. H. Thomas Co.	Philadelphia, Pa.	9766-C
Connectors, O-ring, stainless steel, bore 1.98 mm	Kontes Glass Co.	Vineland, N. J.	a
O-rings, Buna-N, i.d. 6.35 mm o.d. 9.5 mm, w 1.6 mm	A. Trostel Packings, Ltd.	Lake Geneva, Wis.	ARP 568 A-010
Pinch clamps, size 7 A, with locking device, fork opening 4.5 mm	Kontes Glass Co.	Vineland, N. J.	a
Hosecock clamps	Fisher Scientific Co.	Springfield, N. J.	5-847
Three hose end stopcocks for 3.17 mm i.d. tubing, with MS12 spring clips	Beckton, Dickinson and Co.	Rutherford, N. J.	3165
Female Luer-Lok tip to hose end	Beckton, Dickinson and Co.	Rutherford, N. J.	9040 (5200 A)
When autoclaving is necessary, some of the above listed materials can be advantageously substituted by the following:			
Viton tubing for Masterflex pump i.d. 4.76 mm, o.d. 9.5 mm	Cole Parmer	Chicago, Ill.	6412
S-50-HL autoclavable Tygon tubing for all other connections i.d. 3.17 mm, o.d. 6.35 mm	A. H. Thomas Co.	Philadelphia, Pa.	9766-D
O-rings, Viton, i.d. 6.35 mm, o.d. 9.5 mm, w 1.6 mm	A. Trostel Packings, Ltd.	Lake Geneva, Wis.	ARP 568 A-010

[a] Article available on request. Will be listed soon as standard catalog item.

connectors, stopcocks, etc. should be chosen as to preserve lamellar flux of fluids and to insure permanent tightness.

The proposed system must be easily disassembled for the purpose of cleaning. A large number of experiments involving continuous reconnection of the different components should not affect the hermeticity of the system. As a desired feature for certain types of work, any parts in contact with solutions should be autoclavable for sterilization.

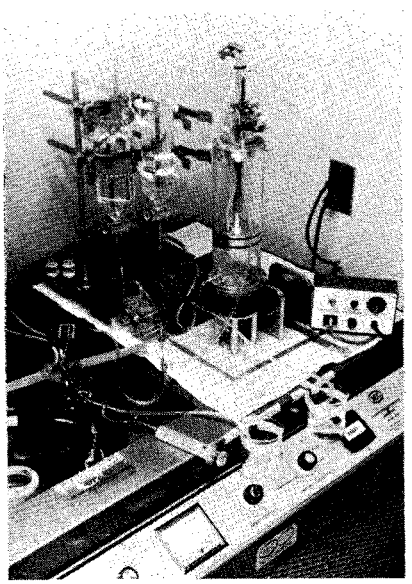

FIG. 1. Zonal ultracentrifugation system mounted on top of an IEC B20 centrifuge adapted to accept the IEC B XIV zonal rotor. Rotor and upper seal bearing are shown outside of the centrifuge barrel for convenience.

Many possible designs meeting these basic requirements were tried in our laboratory. Gravity force alone, positive or negative air pressure, metering and peristaltic pumps, and motor-driven piston devices as a means for the handling of the solutions were also tested. The information gained allowed us to assemble the multipurpose zonal ultracentrifugation system illustrated in Fig. 1. A list of the commercially available components and their sources is given in Table I.

The system is operated in a manner patterned after the suggestions of the group of Anderson *et al.*[1] A procedure will be given for the preparation of the hollow rotor for high speed centrifugation, as illustrated in Fig. 2, and further collection of the gradient containing the banded particles. This procedure is applicable only to removable seal-type rotors.[4] The setup, however, can be adapted to continuous flow centrifugal capabilities with certain modifications. The operative manual, divided in a number of phases for convenience, can be easily followed by means of the schematized layout shown in Fig. 3.

PHASE 1

After the centrifuge and the zonal head are prepared and cooled, the rotor is accelerated up to the working speed

[4] N. G. Anderson, D. A. Waters, W. D. Fisher, G. B. Cline, C. E. Nunley, L. H. Elrod, and C. T. Rankin, Jr., Anal. Biochem. **21**, 235 (1967).

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://scitationnew.aip.org/termsconditions. Downloaded to IP: 35.8.11.3 On: Thu, 28 Jan 2016 11:41:14

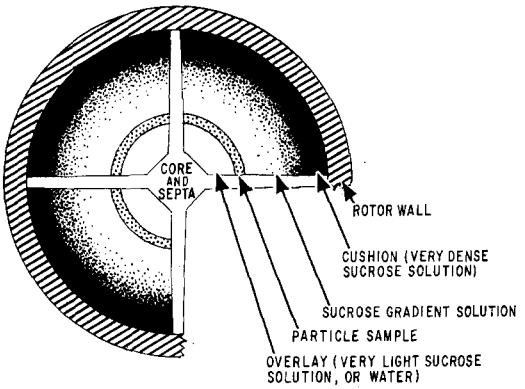

FIG. 2. Top view of zonal rotor ready for high speed centrifugation, showing the relative position of cushion, gradient, sample, and overlay solutions. The drilled core and edge lines have been omitted.

for loading and unloading operations (usually 1500 to 3000 rpm) and the removable seal with its own cooling circuit is placed in position. A detachable stand with the standpipes containing the heavy and the light sucrose solutions, precooled for several hours, is placed on top of the centrifuge, and all the connections shown in Figs. 1 and 3 are fastened by means of pinch-clamps.

Dense sucrose solutions have a high calorific capacity and will remain cold for a period long enough to allow the preparation of a cold density gradient solution. In addition, during the introduction of solutions through the rim inlet of the rotor, there is a uniform reorientation of the gradient around its inner surface. The contact of narrow layers of sucrose solution with the rotor results in an efficient thermal interchange and the gradient is rapidly cooled. Cooling jackets for the standpipes or other refrigeration devices were found unnecessary.

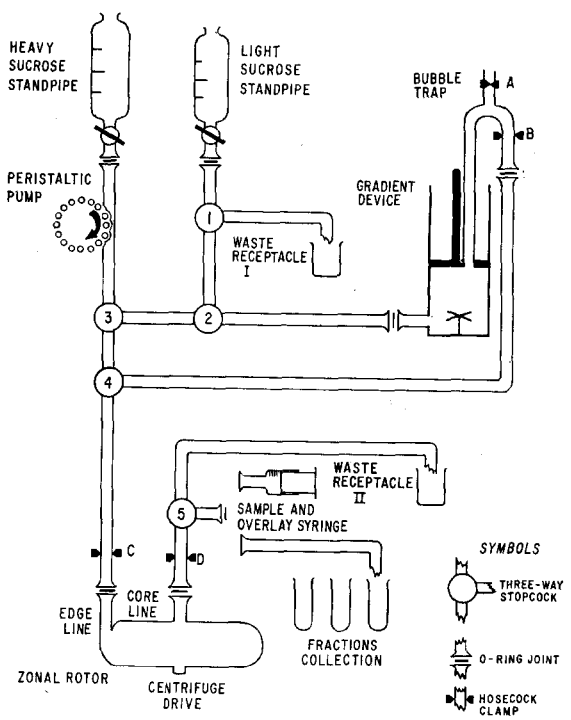

FIG. 3. Schematic representation of the zonal ultracentrifugation system shown in Fig. 1.

PHASE 2

In order to fill the gradient device, clamps A and B are set open and closed, respectively (Fig. 3, opposite of position shown). The diagrams and the operative manual given include the gradient device developed by the author.[5] This apparatus, characterized by variable volume settings and a detachable chamber, is able to provide reproducible exponential gradient solutions of a volume ranging from 100 to 1200 ml. The calculations of the sucrose volumes and concentrations to be used can be made as described by Anderson and Rutenberg.[6] The transparent chamber of the device presented in Fig. 1 was made of cast acrylic resin, which is not autoclavable. A brass chamber (not shown) is used when autoclaving is necessary. Other gradient devices are needed when linear or specially shaped gradients are required.[2] A similar layout may be used for work with any gradient-forming machine, since they all operate on the same principles.[6]

PHASE 3

A tubing pump regulates the introduction of heavy sucrose solution into the mixing chamber of the gradient device through three-way stopcocks 3 and 2. Clamp B is open and the mixed gradient is allowed to flow through three-way stopcocks 4 and to enter the zonal head through the rim inlet. Clamps C and D are open. Displaced air leaves the rotor through the center core line of the rotary seal and through three-way stopcock 5. This line now ends in waste receptacle II. A few drops of solutions will be collected there as the air leaves the rotor. An unusual amount of sucrose solution in waste receptacle II during this operation means cross mixing of lines in the rotary seal, which has to be carefully inspected before and during operation.[1] The delivered amount of heavy sucrose solution can be estimated by means of the graduated marks engraved on the body of the corresponding standpipe, with the correction for the small dead space represented by the hose connections. After delivery of the calculated volume of heavy sucrose solution, the tubing pump is stopped, clamp B is closed, and the gradient device mixer is stopped, in this order.

A suitable speed should be chosen for the preparation of the gradient and simultaneous delivery to the rotor in the described manner. The factors to be considered are the volume of gradient to be fed and the efficiency of the gradient device mixer. The author obtains very repro-

[5] J. E. Paris, Biochim. Biophys. Acta **165**, 286 (1968).
[6] N. G. Anderson and E. Rutenberg, Anal. Biochem. **21**, 259 (1967).

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://scitationnew.aip.org/termsconditions. Downloaded to IP: 35.8.11.3 On: Thu, 28 Jan 2016 11:41:14

ducible gradient solutions of a volume of 500–600 ml by means of his gradient device when the operation lasts for 20 min. The head and pump listed in Table I provide a working pressure of about 2.8 $kg \cdot cm^{-2}$, enough to insure suitable flow rates even in the case of the very viscous sucrose solutions used sometimes as cushion (60–65 wt%). Smaller or larger volumes could require other flow rates and therefore a different head for the pump. Heads covering a wide range of flow rates are commercially available.

PHASE 4

It is customary to keep the volume of the gradient solution smaller than the capacity of the zonal rotor. The remaining space is occupied by a layer of very heavy sucrose solution or "cushion." In many cases, the dense sucrose used for the preparation of the gradient may also serve as cushion, but a third standpipe filled with other sucrose solution may be readily adapted if needed. The cushion is fed to the rim line of the rotor through three-way stopcocks 3 and 4. The tubing pump serves again to regulate the desired flow rate. Although the distance between three-way stopcocks 3 and 4 should be kept very short, it might be noted that some air remains trapped in the area. Such bubbles may be eliminated through three-way stopcock 4 in the direction of the gradient-forming device.

Phase 4 ends when the light end of the gradient, put in motion by the incoming heavy solution, appears from the core line of the rotary seal. In absence of any leakage, the measured amount of solutions delivered should coincide with the known capacity of the zonal rotor, allowing a difference for the small volume of the connecting hoses.

PHASE 5

The cooled sample is prepared for introduction into the rotor and placed in a standard syringe of the required volume. After attachment of the syringe to the system in the position shown in Fig. 1, the sample is forced through three-way stopcock 5 to the core line of the rotor. As the sample enters the zonal head, an excess of cushion solution is displaced from the rotor through three-way stopcocks 4, 3, 2, and 1, and is finally collected in waste receptacle I. This waste receptacle was placed lower than the rotor in order to reduce the pressure necessary for the operation. The distance between the sample-containing syringe and the zonal head is kept as short as possible.

The sample is followed by about 100 ml of overlay, that is, a very light sucrose solution or water destined to displace the sample away from the rotor core. For the introduction of the cooled overlay, a syringe of the required volume is used in the same manner as described for the sample. This will displace more heavy sucrose toward waste receptacle I. After completion of this step, the rotor is ready for the actual centrifugation or ultracentrifugation, and the several solutions will be distributed as shown in Fig. 2.

PHASE 6

Clamps C and D are closed, the removable seal is detached, the rotor is capped, and the centrifuge lid is closed. Centrifugation takes place for the calculated time and speed. During this period the stand with the sucrose-filled standpipes may be temporarily stored in the refrigerator. After the centrifugation, the rotor is decelerated to the loading–unloading speed, the seal put in place atop the uncapped rotor, the standpipes are attached, and clamps C and D are reopened.

PHASE 7

Heavy sucrose solution is pumped to the rim line of the rotor through three-way stopcocks 3 and 4 forcing the gradient with the dispersed particles to leave the zonal head through the core line and stopcock 5. The gradient stream can be fed into a continuous flow analyzer or can be divided into fixed volume portions or "cuts." In this case, a suitable hose connection is attached in place of the sample syringe shown in Fig. 1, and the fine control of the tubing pump is operated so as to deliver the desired volume cuts to a series of refrigerated, graduated test tubes. Although this operation may be facilitated if done by means of an automatic gradient fractionator,[7] graduated test tubes will be found satisfactory for most purposes.

The capabilities of the described zonal ultracentrifugation system were tested in our laboratory for a variety of particle separation experiments. In all cases, experimental determinations of the density of the collected fractions showed that the several manipulations did not result in noticeable changes of the characteristics of the gradient. In our experience, such a system insures a satisfactory, trouble-free performance in the hands of operators not specially trained for the purpose. Some of the suggestions made in this paper will be found useful for the planning of zonal particle separations.

The advice of Dr. David E. Brandes in the preparation of the manuscript is acknowledged. The skillful technical assistance of Robert Schroeder is also acknowledged. This work was supported by Grant No. CA-06518 of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

[7] M. K. Brakke, Anal. Biochem. 5, 271 (1963).

This article is copyrighted as indicated in the article. Reuse of AIP content is subject to the terms at: http://scitationnew.aip.org/termsconditions. Downloaded to IP: 35.8.11.3 On: Thu, 28 Jan 2016 11:41:14