

Sample Re-mixing During Density Gradient Separations with Thermo Scientific Fiberlite F21-8x50y mL Fixed-Angle Rotor

Owen Mitch Griffith, Ph.D.

KEY WORDS

- Density Gradient
- Acceleration/Deceleration Profiles
- Carbon Fiber Rotors
- Superspeed Centrifuges
- Pelleting Studies

Introduction

Fixed-angle rotors are known for having rapid rates of acceleration and deceleration resulting in shorter run times than swinging-bucket rotors. This report investigates the question of whether samples are re-mixed during the rapid deceleration and acceleration in pelleting studies and density gradient methods. Procedures used the Thermo Scientific Fiberlite F21S-8x50y mL fixed-angle carbon fiber rotor with Bioseal technology and the Thermo Scientific Sorvall RC-6 superspeed centrifuge.

In this study, large subcellular organelle fragments were pelleted from a homogeneous solution using rapid deceleration. We first determined whether recovery of low molecular weight materials separated from the lysed cells could be recovered from the meniscus of the sample solution.

In a second experiment, we ascertained whether a discontinuous gradient in the centrifuge tube would be disturbed and whether the separated sample zones at the gradient (sample interface) would be mixed during either acceleration or deceleration.

Procedure

The sample was prepared by Nicole Dyer, Senior Research Scientist, Roche Biosciences, Palo Alto. Classified bacteria cells from a 48-hour cultured cell suspension were harvested and lysed with sodium dodecyl sulfate (SDS) to produce a sample suspension of nucleic acids, cell membranes, proteins and other cellular organelles^{1,2}.

Recovery of Cellular Material at Meniscus

In the first experiment cell membranes, nucleic acids and other cellular organelles were collected as a packed pellet after centrifugation



The Sorvall RC 6 Plus centrifuge



The Fiberlite F21S-8x50y mL fixed-angle carbon fiber rotor

at 49,000 x g for 10 min at 4°C.

A Sorvall superspeed centrifuge and Fiberlite™ F21S-8x50y rotor were used for the density gradient runs. Run times were calculated from the K-factor of the rotor³. Nucleo-proteins and other microsomes were recovered at the sample meniscus located in the top 5 to 10 mL of the supernatant in the centrifuge tube. Amido Schwartz dye and other spectrophotometric determinations were used to identify the nucleic acids and nucleo-proteins. The results of the recoveries were observed from the first four runs, when the rotor was either decelerated slowly or rapidly. (See Table 1.)

Sample Interface Using Discontinuous Sucrose Gradient

The second experiment involved the observation of the sample interface to

check for mixing when using a discontinuous sucrose gradient. A four-step discontinuous gradient consisting of four equal volumes of 10 mL each was formed as described below.

Gradient Preparation

The layers embodied 20% w/w (0.63 M), 30% w/w (0.98 M), 40% w/w (1.375 M) and the sample adjusted with 50% w/w (1.8 M) sucrose concentrations. To facilitate the gradient preparation in the tube:

- the lightest (20%) or first 10 mL layer was injected into the bottom of the tube with a hypodermic needle and syringe
- the second 30% layer was layered below the 20% layer
- next, the third 40% layer was layered below the 30% layer
- finally the sample in the 50% layer was layered below the 40% layer

Experiments	Acceleration	Deceleration	Proteins Recovered
Run #1	Rapid to max speed	Rapid to rest	Mixed in top 20 ml
Run #2	Rapid to max speed	Rapid to 300 rpm, then slow to rest	Mixed in top 10 ml
Run #3	Rapid to max speed	Rapid to 800 rpm, then slow to rest	Mixed in top 5-6 ml
Run #4	Rapid to max speed	Rapid to 1,000 rpm, then slow to rest	Mixed in top 5-6 ml
Membranes Recovered			
Run #5	Slow to 300	rapid to 800 rpm, then slow to rest	Sharp zone observed at the 25% and 40% sucrose interface
Run #6	Slow to 800	rapid to 800 rpm, then slow to rest	Sharp zone observed at the 25% and 40% sucrose interface

Table 1. Schematic of Acceleration and Deceleration Protocols and Results of Proteins/Membranes Recovered

The needle was held as close to the bottom of the centrifuge tube during the layering process of each concentration of the gradient. After the tubes/bottles were capped and loaded into the Fiberlite™ F21-8x50y mL rotor with Bioseal technology, the samples were spun in a Sorvall super-speed centrifuge at 49,000 x g for 90 min at 4°C.

These runs (Table 2, Runs 5 and 6) were done to show recovery of the membranes when slow acceleration and slow deceleration settings were used. The nucleic acids and cellular organelles were pelleted through the 40% sucrose layer, while the membranes were seen as a visible zone at the interface of the 20% and 30% sucrose layers.⁴

Results

Table 1 shows the results of these studies that examine the recovery of cellular material at the meniscus and sample re-mixing with a discontinuous sucrose gradient when different acceleration and deceleration profiles are used during centrifugation.

Conclusion

In view of the above results, it is suggested that when separation by floating techniques are used in fixed-angle rotors, the rotor should be decelerated rapidly to 800 rpm, then slowly to rest. This technique can be used to prevent remixing of a soft sample pellet and the separated components at the meniscus of the sample during rapid deceleration due to

the Coriolis Effect.⁵

In density gradient studies with discontinuous gradients using fixed-angle rotors, slow acceleration rate is required up to 300 rpm, then, the rotor should be rapidly accelerated to the maximum set speed. This initial slow acceleration rate permits the gradient layers to diffuse and become almost linear before separation commences in the centrifuge tube. Therefore, preparing a linear density gradient is not necessary for fixed-angle rotors as is needed in swinging bucket rotors.

During deceleration in density gradient studies, the rotor should be decelerated rapidly to 800 rpm, then slowly to rest. This method serves two purposes. The first is to avert any separated zones at the meniscus of the gradient from being disturbed during deceleration. The second is to prevent disturbance of the already separated zones in the gradient, which would cause a loss of resolution.

Sorvall superspeed centrifuges and Fiberlite carbon fiber rotors can be used as described above to efficiently conduct sample separations without the possibility of disturbing samples during rapid acceleration and deceleration. Furthermore, the F21S-8x50y carbon fiber rotor with Bioseal technology allows for the safest separations at maximal g-force.

Rotors featuring Bioseal technology have been rigidly tested for microbiological containment by the Public Health Laboratory Service, Centre for Applied Microbiological

Research, Porton Down, UK, and shown to be suitable for use with materials up to ACDP Category 3 as categorized by the Advisory Committee on Dangerous Pathogens.

References

- Birnboim, H. C. Doly, J. (1979). A rapid alkaline procedure for screening recombinant DNA. *Nucleic Acid Research*, 7: 1513.
- Maniatis, T., Fritsch, E.F., Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Laboratory.
- Griffith, O.M. (1986). Techniques of preparative, zonal and continuous flow ultra centrifugation. Palo Alto: Spinco Div., Beckman Instruments Inc.
- Hall, K.L. (1983). *Plasma Membranes and Organelles from Plant Cells 55-81*. Hall and Moore (Ed.), London: Academic Press.
- Berman, A.S. (1966). The development of Zonal centrifuges. *Mono-graph*, 21, 41-46. Bethesda: National Cancer Institute.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

North America:
USA / Canada
+1 866 984 0039

Europe:
Austria
+43 1 801 40 0

Belgium
+32 2 482 30 30

France
+33 2 2803 2000

Germany national toll free
08001-536 376

Germany international
+49 6184 90 6940

Italy
+39 02 02 95059 341

Netherlands
+31 76 571 4440

Nordic countries
+358 9 329 100

Russia / CIS
+7 (812) 703 42 15

Spain / Portugal
+34 93 223 09 18

Switzerland
+41 44 454 12 12

UK / Ireland
+44 870 609 9203

Asia:
China
+86 21 6865 4588 or
+86 10 8419 3588

India
+91 22 6716 2200

Japan
+81 45 453 9220

Other Asian countries
+852 2885 4613

Countries not listed:
+49 6184 90 6940 or
+33 2 2803 2000

www.thermo.com/centrifuge

© 2009 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.