

Density Gradient with FIBERLite Fixed Angle Rotor F21-8x50 mL (Sample Remixing)

Owen Mitch Griffith, Ph.D

Fixed angle rotors have been known for having rapid acceleration and deceleration thus resulting in shorter run times than swinging bucket rotors. The question of whether samples are remixed during the rapid deceleration and acceleration in some pelleting studies and density gradient methods is investigated in this report.

Popular fixed angle rotors for High/Superspeed centrifuges, especially the 8 x 50 ml volume metal rotor and the F21-8 x 50 ml light weight carbon fiber rotor, manufactured by FIBERLite Centrifuge Inc. (PTI), were tested for sample mixing during rapid acceleration and rapid deceleration. These rotors generate approximately 50,000 x g at 20,000 RPM with a total sample volume of 400 ml.

In this study we ascertained whether a discontinuous gradient in the centrifuge tube would be disturbed and the separated sample zones at the gradient/sample interface would be mixed during either acceleration or deceleration. This mixing would therefore result in loss of resolution of the separated sample zones. In another experiment we determined whether recovery of low molecular weight materials separated from lysed cells can be recovered from the meniscus of the sample solution, after pelleting of large subcellular organelle fragments from a homogeneous solution during rapid deceleration.

Methods and Materials

The sample was prepared by Mrs. 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 deodecyl sulphate to produce a sample suspension of nucleic acids, cell membranes, proteins and other cellular organelles. (1,2.)

The first study done was to collect cell membranes, nucleic acids and other cellular organelles as a packed pellet in 10 min at 49,000 x g 4 deg. C to be used in the density gradient runs. The run time was calculated from the K-factor of the rotor.(3) The nucleo proteins and other microsomes were recovered at the sample meniscus, the top 5 - 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

The second study was done to observe sample/gradient interface mixing when a sucrose discontinuous gradient was used. A four-step discontinuous gradient, consisting of four equal volumes of 10 ml each was formed.

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 under layered below the 20% layer, followed by the third 40% layer below the 30% layer and finally the sample in the 50% layer was layered below the 40% layer.



FIBERLite F21-8x50 mL rotor

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 the loaded rotor was spun in the centrifuge at 49,000 x g, for 90 min at 4 deg C.

The fifth and sixth runs were done to show recovery of the membranes when slow acceleration and slow deceleration were used before and after the run respectively. 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 the studies when either slow acceleration or slow deceleration was used. These results were compared to studies made with rapid acceleration and rapid deceleration.

Table 1.

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 RPM, then rapid to max speed	Rapid to 800 RPM, then slow to rest	Sharp zone observed at the 25% and 40% sucrose interface
Run #6	Slow to 800 RPM, then rapid to max speed	Rapid to 800 RPM, then slow to rest	Sharp zone observed at the 25% and 40% sucrose interface

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 was used to prevent a soft sample-pellet or the separated components floating at the meniscus of the solution in the tube, from being remixed due to the Coriolis Effect on the sample during rapid deceleration. (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 to avert any separated zones at the meniscus of the gradient from being disturbed during deceleration and the second to prevent disturbance of the already separated zones in the gradient thereby losing resolution.

For Further Information:

Contact us at:

FIBERLite Centrifuge, Inc.
422 Aldo Ave.
Santa Clara, CA 95054
408-988-1103 Tel
408-988-1196 Fax
www.piramoon.com

References

1. Birnboim, H. C. and Doly, J. A rapid alkaline procedure for screening recombinant DNA. *Nucleic Acid Research*, 7: 1513, (1979)
2. Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual*, New York, Cold Springs Harbor Laboratory, (1982)
3. Griffith, O.M., *Techniques of Preparative Zonal and Continuous Flow Ultracentrifugation*, 5th Edition, Beckman Instruments, Spinco Division, Palo Alto, (1986)
4. Hall, K.L., *Plasma Membranes and Organelles from Plant Cells*, 55-81, Edited by Hall and Moore, London, Academic Press, (1983)
5. Berman A.S., *The development of Zonal centrifuges*, Bethesda, MD: National Cancer Institute, Monograph no. 21, 41-46, (1966)