# Application Information

## Protein Separation

# Predicting Protein Separation in Rate Zonal Centrifugation Using the ESP™ Rate Zonal Run Simulation from the Optima eXPert™ Software

*Mei-Ling Chien*
*Beckman Coulter, Inc.*

Rate zonal centrifugation is a common technique for the separation, purification, and isolation of macromolecules in biological research. In this method, samples are overlaid onto a pre-formed gradient (e.g., sucrose) and then centrifuged. Separation is based on the sedimentation coefficient (*s* value) of the molecule, which is in turn determined by the molecular weight, density, size, and shape. In rate zonal experiments, selection of the correct centrifugation time is important because too short a spin is not sufficient to separate the molecules and too long a spin results in pelleting of one or more species.

The ESP™ Rate Zonal Run simulation in the Optima eXPert™ software provides an informative and intelligent way to plan experimental protocols with any user-specified rotor and tube. In this article, separation of lysozyme and aldolase is used as an example to demonstrate the ability of the ESP Rate Zonal Run simulation to predict the amount of time required for separating proteins in a linear sucrose gradient.

## Using the Simulator

The first parameters required for input are the sedimentation coefficients (*s*) of the molecules of interest. The *s* values for lysozyme and aldolase are known to be 1.9 *s* and 7 *s* (Svedberg units, $10^{-13}$ seconds; references 1 and 2), respectively. In cases where the *s* values are unknown, they can be estimated from molecular mass using the calculator–*Estimate Sedimentation Coefficient from Molecular Mass*–also provided in the Optima eXPert software under the *Calculate* menu (Figure 1, reference 3). This calculator can provide estimates for the *s* values of macromolecules such as globular proteins, RNA, and different topological forms of DNA. The *s* values for some common biological particles can be found in reference 5. Once the *s* values are at hand, open the *Simulate* menu and select *ESP Rate Zonal Run.* Input the *s* values (for up to three species), a rotor and a tube, select a sucrose gradient concentration (5 to 20% or 10 to 40%), particle density (1.3 g/mL for proteins, 1.5 g/mL for ribo-

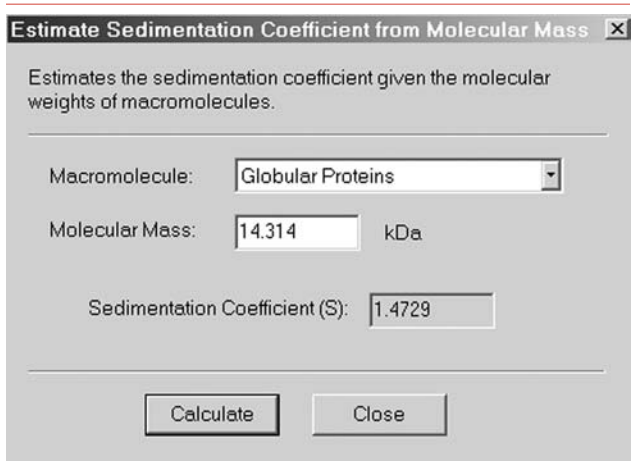


***Figure 1.*** *The "Estimate Sedimentation Coefficient from Molecular Mass" calculator provided in the Optima eXPert software. The molecular mass for lysozyme, 14.314 kDa (reference 4), was entered as an example and the output* s *value from the calculator was 1.4729, which is close to 1.9.*

BECKMAN COULTER

somes containing RNA and 1.7 g/mL for DNA) and temperature (5°or 20°C) and then click *Simulate Protocol.*

To simulate lysozyme and aldolase separation in a rate zonal experiment, *s* values of 2 and 7 (the closest integer values) were entered. The rotor and tube selected were SW 32 Ti and 38.6 mL Open Top, Beckman Coulter PN 326823. The gradient selected was a 5 to 20% linear sucrose gradient, the particle density 1.3 g/mL, and the temperature 5°C. The total simulated elapsed time was 80 hours 30 minutes when the faster band (aldolase) reached the bottom of the tube. In order to obtain good separation and prevent the leading band from pelleting, as recommended, the scroll bar was adjusted so that the bottom band was positioned at around 2/3 of the distance down the tube. This corresponded to an elapsed time of 48 hours (Figure 2).

## Experimental Verification

To verify the simulation results, a rate zonal experiment was performed in which 1 mL of sample containing 4 mg of lysozyme and 10.5 mg of aldolase (both from Sigma) in water was overlaid on a 5 to 20% linear sucrose gradient (36 mL, made by using a two-chambered linear gradient maker, Hoefer Scientific Instruments) in an Ultra-Clear™ tube from Beckman Coulter, PN 344058 (with the same size and volume as the tube used for the simulation, Beckman Coulter PN 326823). The tubes were centrifuged in the SW 32 Ti rotor at 32,000 rpm, 5°C, in an Optima™ L-100 XP ultracentrifuge. After centrifugation for 48 hours, the tubes were removed and fractions collected from the bottom of the tube using a Beckman Coulter Fraction Recovery System, and the absorbance at 280 nm was measured using a Beckman Coulter spectrophotometer with a micro flow cell. The results, shown in Figure 3, demonstrate that the simulation software successfully predicted the separation of the two test proteins using the selected parameters.

In conclusion, the *ESP™ Rate Zonal Run* simulation is a useful tool for predicting how long it will take to separate macromolecules or particles using any rotor and tube combination in a 5 to 20% or 10 to 40% linear sucrose gradient. This saves researchers' time, materials, and labor for pilot experiments.

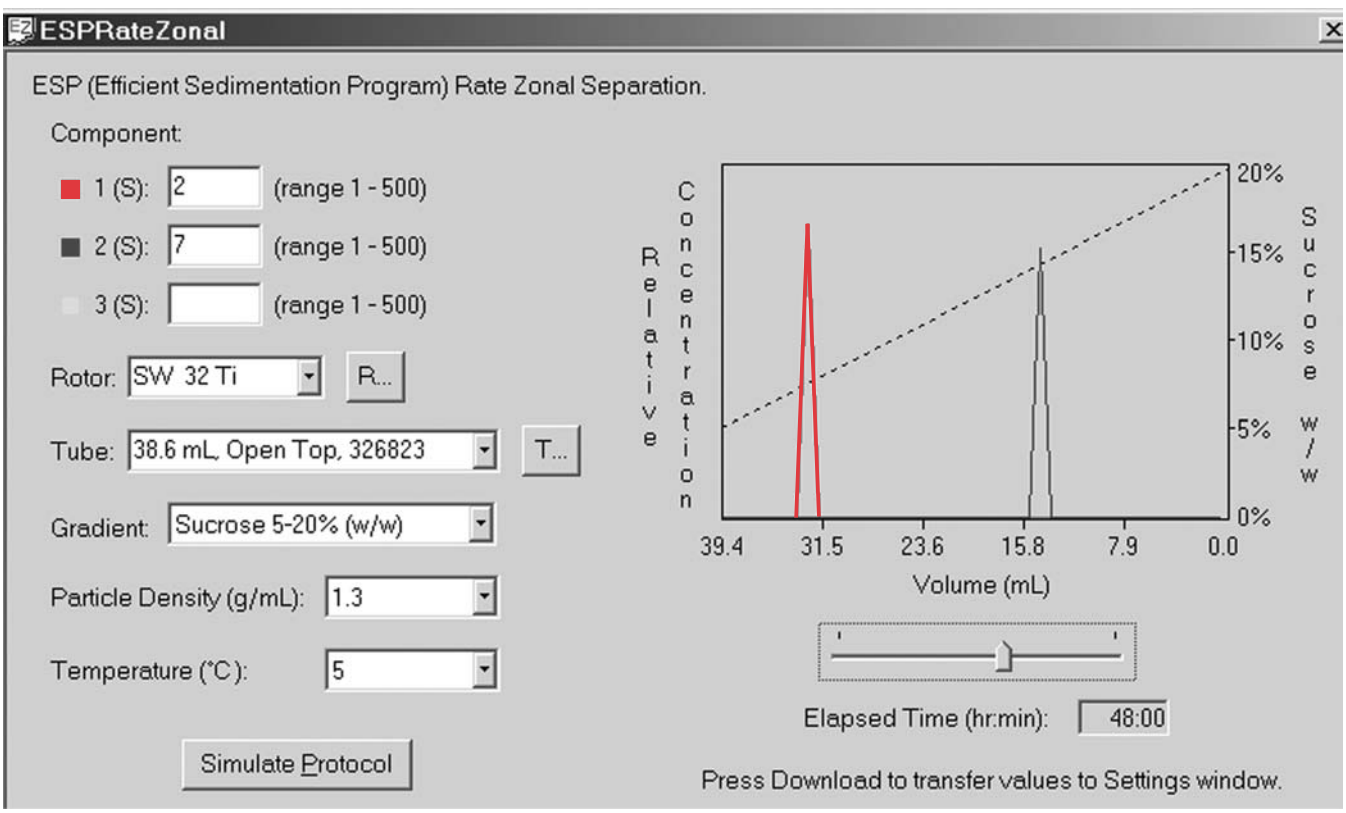


***Figure 2.*** *ESP Rate Zonal simulation for lysozyme (red,* s=*2) and aldolase (black,* s=*7) separation.*

## References


1. van Holde, K. E. *Physical Biochemistry.* 2nd edition, Chapter 5. Englewood Cliffs, New Jersey: Prentice-Hall, Inc, 1985.
2. Rowe, A. J., Khan, G. M. Determination of corrected sedimentation coefficient at different temperatures using the MSE analytical ultracentrifuge. *Analytical Biochemistry 45*, 488-497 (1972)
3. Rickwood, D., Editor. *Centrifugation: A Practical Approach* (2nd edition), p. 133. Oxford: IRL Press, 1984.
4. Gill, S. C., von Hippel, P. H. Calculation of protein extinction coefficients from amino acid sequence data. *Analytical Biochemistry 182*, 319-326, 1989.
5. Griffith, O. M. Techniques of Preparative, Zonal, and Continuous Flow Ultracentrifugation. *DS-468H*, Beckman Instruments, Inc.


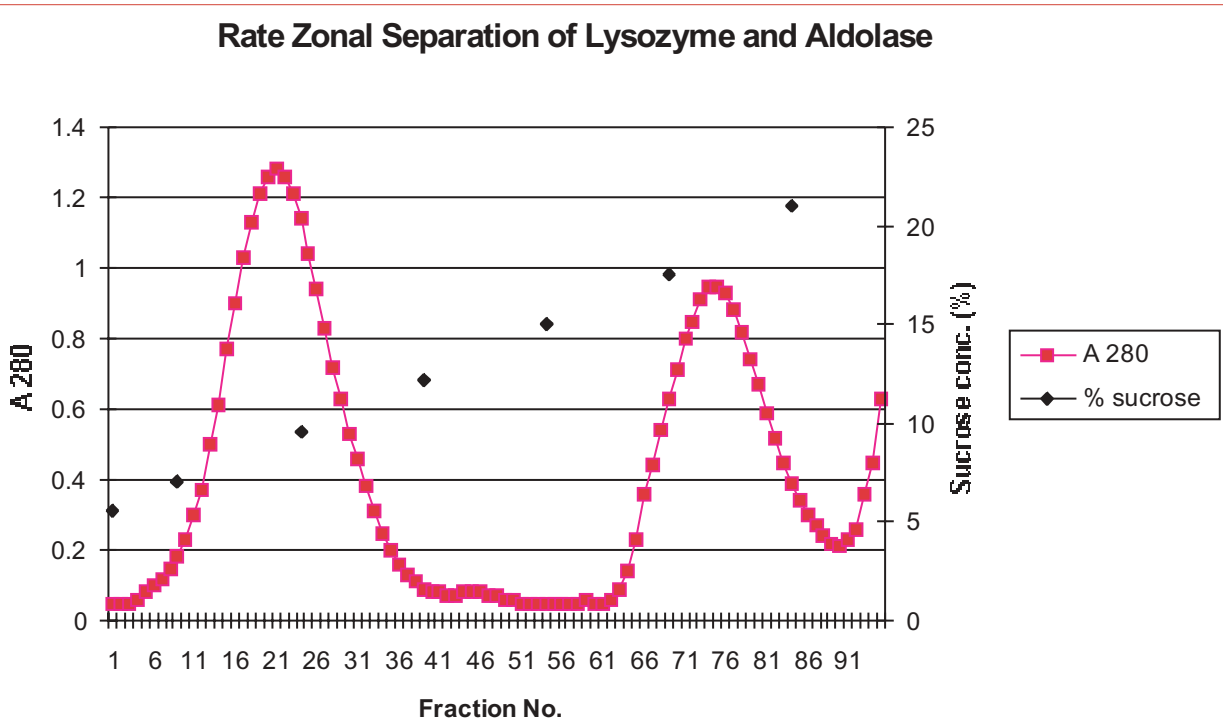


***Figure 3*** *The results of rate zonal separation of lysozyme (left peak) and aldolase (right peak) in a 5 to 20% linear sucrose gradient in the SW 32 Ti rotor centrifuged at 32,000 rpm, 5°C, for 48 hours.*

*All trademarks are the property of their respective owners.*



*Developing innovative solutions in genetic analysis, drug discovery, and instrument systems.*

Innovate SIMPLIFY Automate

Beckman Coulter, Inc. • 4300 N. Harbor Boulevard, Box 3100 • Fullerton, California 92834-3100
Sales: 1-800-742-2345 • Service: 1-800-551-1150 • Telex: 678413 • Fax: 1-800-643-4366 • www.beckmancoulter.com

**Worldwide Life Science Research Division Offices:**
**Australia** (61) 2 9844-6000 **Canada** (905) 819-1234 **China** (86) 10 6515 6028 **Eastern Europe, Middle East, North Africa** (41) 22 994 07 07 **France** 01 49 90 90 00 **Germany** (89) 358700 **Hong Kong** (852) 2814 7431 / 2814 0481 **Italy** 02-953921 **Japan** 03-5404-8359 **Mexico** 525-605-77-70 **Netherlands** 0297-230630 **Singapore** (65) 6339 3633 **South Africa/Sub-Saharan Africa** (27) 11-805-2014/5 **Spain** (34) 91 3836080 **Sweden** 08-564 85 900 **Switzerland** 0800 850 810 **Taiwan** (886) 2 2378 3456 **Turkey** 90 216 309 1900 **U.K.** 01494 441181 **U.S.A.** 1-800-742-2345

B2002-5370 LC 10K

Printed in U.S.A. on recycled paper.