Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Transport Phenomena in Zonal Centrifuge Rotors. XII. Dispersion in Reorienting Self-Generating Density Gradients

H. W. Hsu[a]; J. N. Brantley[bc]; J. P. Breillatt[bd]
[a] DEPARTMENT OF CHEMICAL, METALLURGICAL AND POLYMER ENGINEERING, THE UNIVERSITY OF TENNESSEE, KNOXVILLE, TENNESSEE [b] OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE [c] Division of Environmental Sciences, Oak Ridge National Laboratory, [d] Engineering Experiment Station, E. I. du Pont de Nemours & Co., Wilmington, Delaware

To cite this Article Hsu, H. W. , Brantley, J. N. and Breillatt, J. P.(1979) 'Transport Phenomena in Zonal Centrifuge Rotors. XII. Dispersion in Reorienting Self-Generating Density Gradients', Separation Science and Technology, 14: 1, 69 — 77
To link to this Article: DOI: 10.1080/01496397908057145
URL: http://dx.doi.org/10.1080/01496397908057145

SEPARATION SCIENCE AND TECHNOLOGY, 14(1), pp. 69–77, 1979

NOTE

Transport Phenomena in Zonal Centrifuge Rotors. XII. Dispersion in Reorienting Self-Generating Density Gradients*

H. W. HSU†

DEPARTMENT OF CHEMICAL, METALLURGICAL AND POLYMER ENGINEERING
THE UNIVERSITY OF TENNESSEE
KNOXVILLE, TENNESSEE 37916

J. N. BRANTLEY‡ and J. P. BREILLATT§

OAK RIDGE NATIONAL LABORATORY¶
OAK RIDGE, TENNESSEE 37830

Abstract

Dispersion in reorienting self-generating gradient systems for zonal centrifugations was investigated. Dispersions in experimental runs for K-III and J-I rotors were analyzed and compared with previously published analytical results. Various factors that contributed to a sample band broadening due to dispersion are discussed and evaluated. It is concluded that a control of rotor acceleration and deceleration may not be important.

*The work was performed while the authors were with the Molecular Anatomy (MAN) program, Oak Ridge National Laboratory, which was supported by the National Cancer Institute, the National Institute of General Medical Sciences, the National Institute of Allergy and Infectious Diseases, and the U.S. Atomic Energy Commission.

†To whom correspondence should be addressed.

‡Present address: Division of Environmental Sciences, Oak Ridge National Laboratory.

§Present address: Engineering Experiment Station, E. I. du Pont de Nemours & Co., Wilmington, Delaware 19808.

¶Oak Ridge National Laboratory is operated by Union Carbide Corporation Nuclear Division under contract W-7405-eng-26 for the U.S. Department of Energy.

INTRODUCTION

In our mathematical analysis of the area of isodensity surface in reorienting a density gradient system in a zonal rotor with core but without septa configuration, we have shown (*1*) that the dispersion of sample layers in centrifugation is independent of the rate of acceleration in the start-up of a rotor. The dispersion of sample layers (the resolution) depends on the configuration of a rotor and loading levels. With a given rotor at a given loading level, a loss of resolution due to dispersion from the changes of interfacial area is a constant. In this note we have evaluated those dispersion constants for K-III and J-I rotors experimentally and verified our previous analysis.

DISPERSION IN REORIENTING DENSITY GRADIENT

A band-broadening effect in zonal centrifugation is due to a complicated phenomenon consisting of an interaction of diffusion, sedimentation, and shearing forces resulting from interfacial area changes, etc. We combined all of the contributing factors and termed it the dispersion coefficient (*2*).

During the gradient reorientation, from rest to a stable orientation in a high centrifugal force field, the shearing forces occurring in a liquid confined in a closed cylinder will cause an increase in dispersion of a reorienting gradient system. The dispersion coefficient contributed from reorientation, δ, may be written as

$$\delta = \frac{dS}{dt}\left[\frac{\text{area}}{\text{time}}\right] \tag{1}$$

In a given time period the total dispersion due to reorientation shearing forces by changing in isodensity interfacial area is

$$\sigma = \int_0^t \delta\, dt = \int_0^t \frac{dS}{dt}\, dt = S(t) - S(0) \tag{2}$$

An isodensity interfacial area approaches a constant value (completely reoriented) after a certain rotational speed for a given liquid loading level has been reached. A rotational speed, rpm $= 60\omega/2$, is directly proportional to an angular velocity, $\omega = \sec^{-1}$. Thus Eq. (2) may be rewritten

$$\sigma = \int_0^t \frac{dS}{dt}\,dt = \int_0^{t_c} \frac{dS}{dt}\,dt + \int_{t_c}^{t} \frac{dS}{dt}\,dt = S(t_c) - S(0)$$

$$= \int_0^{w_c} \frac{dS}{d\left(\frac{1}{\omega}\right)}\,d\left(\frac{1}{\omega}\right) + \int_{w_c}^{w} \frac{dS}{d\left(\frac{1}{\omega}\right)}\,d\left(\frac{1}{\omega}\right) = S(\omega_c) - S(0) \qquad (3)$$

in which t_c is the time required to reach a completely reoriented paraboloid configuration, and ω_c is the angular velocity at which the paraboloid configuration is completely reoriented. From Eq. (3), one may see that $S(\omega_c)$ is a function of rotor configuration and liquid loading level only.

EXPERIMENTAL PROCEDURE AND ANALYSIS

Equations (2) and (3) were used in the analysis of experimental runs using K-III and J-I rotors. Sample distributions in terms of UV absorbances after a specified acceleration and deceleration are shown in Figs. 1 and 2 for K-III and J-I rotors, respectively. The dimensions of these two rotors are listed in Table 1.

The 435-sec run for the K-III rotor was made by the following procedures: (1) the rotor was filled with 1 to 34% sucrose gradient solution from the bottom of the rotor by pumping, (2) a dense sucrose solution (66%) was pumped into the rotor from the bottom to remove a 300-ml fraction of the gradient solution from the top of the rotor and stored in a funnel, (3) the dense sucrose solution was pumped into the rotor and the 300 to 500 ml fraction of the gradient solution from the top of the rotor was discarded, (4) a 50-ml sample of yeast RNA (0.62 mg/ml) in 10% sucrose solution was loaded into the rotor from the top, (5) the 0 to 300 ml fraction of gradient solution was returned through a funnel over the sample layer, (6) the rotor was started from rest to 2000 rpm in 45 sec, and, finally, (7) the rotor was brought to rest from 2000 rpm in 390 sec.

In the 465-sec run the following procedure was followed: (1) the 0 to 300-ml fraction of 1 to 34% sucrose gradient solution was pumped into the rotor from the bottom, (2) the 50-ml sample was loaded, (3) the 300 to 500 ml fraction of the gradient solution was discarded from the gradient pumping line, (4) the rotor was then filled with the rest of the sucrose gradient solution, and (5) the rotor was started to accelerate to 2000 rpm in 45 sec and from 2000 rpm to a complete stop in 420 sec.

For the 1440-sec run the gradient solution and sample were loaded into the rotor exactly the same as for the 465-sec run. The rotor was

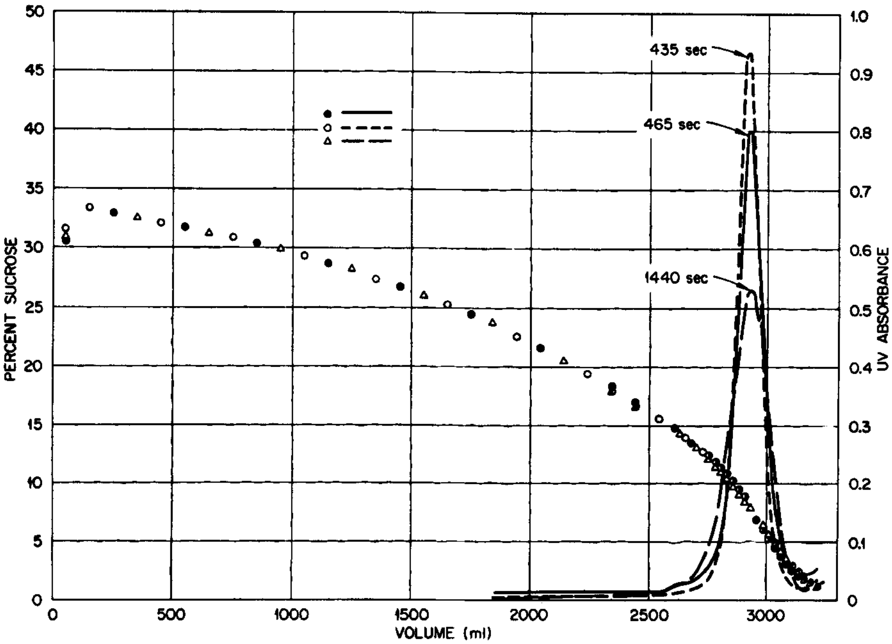

FIG. 1. Absorbance curves of yeast RNA in K-III rotor runs.

carefully accelerated to 500 rpm in 250 sec, then further accelerated to 2000 rpm in 375 sec, and decelerated to a full stop in 13 min, 35 sec.

In all three runs the sample was loaded in an exact location between 2870 ml of dense fraction and 300 ml of light fraction of the gradient solution to give the reduced liquid loading level at $0.876 \leq \alpha \leq 0.891$ [where α = (liquid loading height)/(height of rotor)]. In the 465- and 1440-sec runs, the sample was pumped from the bottom; therefore, the sample has been moving along the rotor from the bottom to that loading level.

In Fig. 2, the results of experimental runs on the J-I rotor are presented. Runs A, B, and E were made by filling the rotor from the bottom with 400 ml of 0 to 34.0% sucrose gradient solution, 10 ml of yeast RNA sample solution (2 ml containing 1.22 mg of yeast RNA in 8 ml of 10% sucrose solution), and 390 ml of 35.5 to 46.4% sucrose gradient solution. In Run A the filled solution was set in rest for 130 sec, and then the rotor was unloaded. In Run B the rotor was set to start from rest to 4000 rpm in 10 sec and decelerated to rest in 120 sec. Run E was made by careful

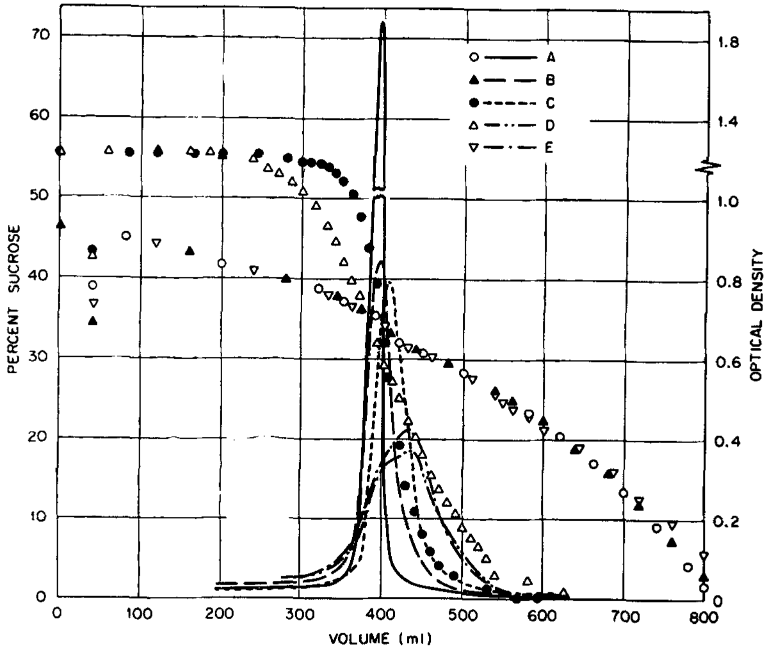

FIG. 2. Absorbance curves of yeast RNA in J-I rotor runs.

TABLE 1

Dimensions of Rotors

Dimension (cm)	K-III	J-I
Height, H	75.08	38.338
Rotor inside diameter, R_w	13.22	8.890
Core diameter, R_c	10.90	7.184

control of acceleration and deceleration of the rotor. The controlling sequences of the rotor were as follows: (1) the initial acceleration of the rotor from 0 to 500 rpm in 4 min, (2) the acceleration from 500 to 2000 rpm in 7 min, (3) the acceleration from 2000 to 4000 rpm in 5 min, (4) the deceleration from 4000 to 2000 rpm in 5 min, (5) the deceleration from 2000 to 500 rpm in 7 min, and (6) the final deceleration from 500 rpm to a complete stop in 4 min. The entire run was completed in 1920 sec.

Runs C and D were made in step gradient solutions by loading the rotor first with 400 ml of water, then adding the 10 ml of the sample, and finally adding the 390 ml of the 55.3% sucrose solution. In Run C the rotor was run exactly as that of Run B for 120 sec, while Run D was made as Run E for 1920 sec. In all five runs the reduced liquid loading levels were between $0.500 \le \alpha \le 0.513$.

From Figs. 1 and 2, one sees that a sample band width is sharper when the rotor accelerates and decelerates at higher speeds. For both types of rotors the band spreading is less for a shorter period of operation time.

In order to gain an insight into a band spreading or a band dispersion in reorienting gradient systems, the second moment and dispersion coefficient in each experimental run were evaluated and are presented in Table 2. Dispersion coefficients were obtained from the second moment, $\langle x^2 \rangle$, by Einstein's formula (*3*)

$$\langle x^2 \rangle = 2Dt \qquad (4)$$

in which D is the dispersion coefficient contributed by molecular diffusion due to the concentration gradient and shearing forces in changes of isodensity area due to the gradient reorientation and other dynamic factors (such as vibration and fluctuation in the rotor speed) and t is the actual measured time. From Eqs. (1) and (2), one also sees that the total dispersion σ defined in Eq. (2) is equal to the second moment defined in Eq. (4). Thus one has

$$\sigma = \langle x^2 \rangle \qquad (5)$$

TABLE 2

Relative Second Moments and Dispersion Coefficients from K-III and J-I Rotor Runs

Run	Second moment $\langle x^2 \rangle$ (cm^2)	Dispersion coefficient, $D \times 10^2$ (cm^2/sec)
	J-I Rotor: Scaling Factor $= 1.983 \times 10^4$	
A (120 sec)	16.188	6.226
B (120 sec)	16.234	6.244
C (120 sec)	16.369	6.296
D (1920 sec)	16.503	4.298
E (1920 sec)	16.427	4.278
	K-III Rotor: Scaling Factor $= 2.696 \times 10^6$	
435 sec	42.206	4.851
465 sec	42.256	4.544
1440 sec	42.268	1.467

The result of Run A for the J-I rotor shows the dispersion due to loading and unloading and molecular diffusion of the sample in gradient solution for a 120-sec period. The difference between Runs B and A may be interpreted as the dispersion due to the gradient reorientation and an increase in molecular diffusion resulting from an increase in interfacial area during the reorientation. The difference in total dispersion between Runs B and A is 0.0467 cm^2.

It is important to point out that the numerical values presented in Table 2 are relative values. The optical absorbances or optical densities given in Figs. 1 and 2 are all relative values which have been scaled to a reference value given as a unit value. For a practical estimation of results presented in Table 2, one has to obtain a scaling factor for K-III and J-I rotors. In this attempt we have used the results presented in Figs. 2 and 3 (see Ref. *1*) in conjunction with the value 0.0467 cm^2 just obtained. The values of β, the radius ratio between the core and inside wall of the rotor, for the K-III and J-I rotors are 0.825 and 0.808, respectively. The reduced total dispersion evaluated for Run B ($\alpha = 0.500$, $\beta = 0.808$) is 43.0. The annular cross-sectional area of the J-I rotor is 21.54 cm^2. Therefore, the estimated dispersion due to reorientation in Run B is 926 cm^2. The molecular diffusion coefficient of yeast RNA to sucrose solution is estimated roughly to be 2.6×10^{-7} cm^2 sec. Therefore, the dispersion due to a molecular diffusion in a 120-sec run is negligible compared to the magnitude of reorientation dispersion. The scaling factor for the J-I rotor is obtained by the method of proportion which gives 1.983×10^4. The difference between Runs E and B gives dispersion due to the difference in acceleration or deceleration and other dynamic factors causing dispersions. The dispersion arising from those sources totals 0.1927 relative units.

In our previous experimental investigations we used the technique of birefringence where a milling yellow dye solution was selected as the optical solution to study two-dimensional transient flow-pattern and shear-stress distributions (*4*). We have found that changing the rate of acceleration or deceleration produces no noticeable effect in any flow patterns, shear-stress distributions, or their magnitudes during transient periods for a given rotor. This experimental finding is in agreement with the analytical analysis presented in Ref. *1*. Therefore, we would like to stress that, in an improvement of resolution for a separation task, a control of rotor acceleration or deceleration does not contribute to the improvement. As demonstrated in Eq. (2), a dispersion arising from a gradient reorientation is a constant for a given rotor and for a given loading level. The magnitude of dispersion due to reorientation is much smaller than that due to loading, unloading, or to the fluctuation in rotor speed or vibration in a rotating system.

A comparison between Runs B with C and Runs E with C demonstrates that a smooth built-in gradient system offers a better result than a step gradient system. A diffusion coefficient is proportional to its concentration difference. In a step gradient system, the concentration difference between a sample zone and the gradient solution is higher than in a built-in gradient system.

In the K-III runs, we attributed the difference in dispersion between a 435-sec run and a 465-sec run to the rate at which the sample moves from the bottom of the rotor to the loading level. Since they were both accelerated in the same way, just the difference in time to bring the rotor to a full stop (which may be categorized as being within an experimental error) was probably the cause of the slight difference in dispersion.

With a limited amount of information, we extrapolated runs of 1440 and 465 sec to zero and subtracted the dispersion due to sample moving; we thus determined the dispersion due to loading and unloading in the K-III rotor to be 42.2 reduced units. Then using Fig. 4 in Ref. *1*, the scaling factor of the K-III rotor was estimated to be 2.7×10^5. Therefore, it is concluded that dispersion due to loading and unloading is the major factor in loss of resolution.

DISCUSSION

From the foregoing analysis of experimental results we would like to make the following suggestions: One should run a rotor as fast as possible in the start-up to reduce the operating time. The band broadening in gradient reorienting systems, the resolution losses due to reorientation are much smaller than those due to loading and unloading. The control of rotor acceleration or deceleration is a very difficult task; since the resolution loss due to this factor is negligible, control is unnecessary. Furthermore, at high speeds the fluctuation of a rotor speed is less than that at low speed, and smooth operation is easier to attain.

Improvement of the zonal centrifuge should be directed to an improvement in the loading and unloading method to reduce the dispersion contributed by this operation. Changing of a pumping system to a gravitational loading method by loading a gradient solution from a dense to a light fraction from the top may be worthwhile.

Acknowledgments

The authors express their appreciation to Dr. N. G. Anderson, a former director of the Molecular Anatomy Program, Oak Ridge National Laboratory, for his encouragement during this work. The partial financial support of NSF Grant, ENG 72–04241 A01, to H.W.H. is gratefully acknowledged.

REFERENCES

1. H. W. Hsu, *Sep. Sci.*, *8*(5), 537 (1973).
2. R. K. Genung and H. W. Hsu, *Ibid.*, *7*(3), 249 (1972).
3. A. Einstein, *Ann. Phys.*, *17*, 549 (1905).
4. H. D. Pham and H. W. Hsu, *Ind. Eng. Chem., Process Des. Dev.*, *11*, 556 (1972).

Received by editor April 24, 1978