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NASA Technical Memorandum

NASA TM-82561

COIL PLANET CENTRIFUGATION AS A MEANS FOR SMALL PARTICLE SEPARATION

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November 1983

National Aeronautics and Space Administration

George C. Marshall Space Flight Center

TECHNICAL REPORT STANDARD TITLE PAGE

1. REPORT NO.	2. GOVERNMENT ACCESSION NO.	3. RECIPIENT'S CATALOG NO.
NASA TM-82561		

4. TITLE AND SUBTITLE	5. REPORT DATE
Coil Planet Centrifugation as a Means for Small Particle Separation	November 1983
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT #
Frederick T. Herrmann	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. WORK UNIT NO.
George C. Marshall Space Flight Center Marshall Space Flight Center, Alabama 35812	11. CONTRACT OR GRANT NO.
	13. TYPE OF REPORT & PERIOD COVERED
12. SPONSORING AGENCY NAME AND ADDRESS	Technical Memorandum
National Aeronautics and Space Administration Washington, D.C. 20546	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

Prepared by Space Science Laboratory, Science and Engineering.

16. ABSTRACT

The coil planet centrifuge uses a centrifugal force field to provide separation of particles based on differences in sedimentation rates by flow through a rotating coiled tube. Three main separations are considered:

1) Single phase fresh sheep and human erythrocytes.

2) Single phase fixed sheep and human erythrocytes.

3) Electrophoretically enhanced single phase fresh sheep and human erythrocytes.

17. KEY WORDS	18. DISTRIBUTION STATEMENT
Coil planet centrifuge Cell sedimentation rates Electrophoresis Erythrocytes Single and double phase systems	Unclassified – Unlimited Frederick T. Herrmann

19. SECURITY CLASSIF. (of this report)	20. SECURITY CLASSIF. (of this page)	21. NO. OF PAGES	22. PRICE
Unclassified	Unclassified	17	NTIS

MSFC - Form 3292 (May 1969)

For sale by National Technical Information Service, Springfield, Virginia 22151

ACKNOWLEDGMENT

The author wishes to acknowledge the aid given by Dr. Yoshiro Ito of the National Institutes of Health at Bethesda, Maryland, in the loan of his coil planet centrifuge and the use of sketches contained in this paper.

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INTRODUCTION

The coil planet centrifuge, as originally designed by Ito [1], is a mechanical process of bringing about separation of macromolecules such as amino acids [2] and small particles such as erythrocytes. Particles are separated on the basis of sedimentation differences for both single phase and double phase systems. In this paper the performance of the coil planet centrifuge is explored experimentally using a single phase particle suspension. The specific particles considered here include both fresh and fixed sheep and human erythrocytes in isotonic buffered saline solution. Another topic considered in this paper is electrophoretic enhancement of single phase erythrocyte separations.

II. APPARATUS

A cross-sectional view through the central axis of the coil planet centrifuge is shown in Figure 1. A continuous ptfe teflon tube, 19 m in length, helically wrapped 600 turns about steel tubing forms the column. The use of motors I and II allows independent adjustment of centrifugal field and coil rotation.

Various types of coil planet centrifugation schemes are shown in Figure 2. The separation schemes show the orientation and motion of the column. The separation schemes are divided into three modes of planetary motion. The mode of non-synchronous coil planet centrifugation discussed will be that of scheme VI. In scheme VI the coil is moved away from the central axis and undergoes a non-synchronous planetary motion.

A functional diagram showing the coil planet centrifuge and its associated peripheral hardware is contained in Figure 3. Absorption peaks and retention times were recorded using a LKB UV detector linked with a HP 9835 Data Acquisition System. Particle collection was accomplished using a LKB Fraction Collector. Cell counts and size distribution were determined using a Coulter ZBI cell counter and C1000 channelizer.

III. MATERIALS AND METHODS

A. Size Distribution and Density Data for Fresh and Fixed Human and Sheep Erythrocytes

Fresh sheep and human erythrocytes were chosen as model particles because of their relative uniformity and availability. All cells were washed three times in isotonic phosphate buffer before their use or analysis. The buffy coat was removed from the fresh samples after the first wash. All formaldehyde-fixed cells were prepared from whole blood according to the procedure described by Nordt [3]. Fixed cells were also washed three times in isotonic phosphate buffer before use or analysis.

Mean erythrocyte specific gravity as reported in the "Handbook of Biological Data" [4] is 1.093 (1.089 to 1.097) for man and 1.084 (1.080 to 1.087) for sheep. Fixed sheep and human erythrocyte specific gravities were confirmed by density gradient centrifugation of erythrocytes in a ficoll linear density gradient. Ficoll mixtures used were 10% w/w and 30% w/w with specific gravities of 1.035 and 1.115, respectively. The cells were layered on top of the gradient preparation and spun at 14,000 rpm for 40 min. Specific gravities as calculated from sedimentation depth measurements are 1.076 for fixed sheep and 1.082 for fixed human erythrocytes.

Erythrocyte volumes reported in the Handbook of Biological Data are given as 87 μm^3 (70 to 104) for man and 31 μm^3 (30 to 32) for sheep. Volume distributions of fixed and fresh erythrocytes were obtained using a Coulter Accucomp Particle Analysis System. Size distribution data is given in Figures 4 through 7.

B. Single Phase Fresh Sheep/Human Erythrocyte Separation

Sheep and human erythrocytes were prepared from EDTA-treated, fresh blood by washing with buffered saline solution three times, repeating centrifugation, and decanting the supernatant. The final erythrocyte suspensions were adjusted to a hematocrit of 40 percent. Equal volumes of human and sheep erythrocyte suspensions were combined.

After the column was filled with isotonic buffered saline solution [5], the sample mixture (0.2 ml) was injected into the sample port. The column was eluted (9.6 ml/hr) with buffered saline solution. The column was rotated counterclockwise around the major axis at 750 rpm (cc). Minor axis column rotation was set at 4 rpm (cc). The column outlet was continuously monitored with an LKB Uvicord III detector set at a wavelength of 200 nm and fractions were collected in test tubes using an LKB 17000 fraction collector. All fractions were analyzed using the Coulter ZBI and C1000 system.

C. Single Phase Fixed Sheep/Human Erythrocyte Separation

The experimental procedure used for this separation was identical to that used in Section B.

D. Electrophoretically Enhanced Single Phase Fresh Sheep/Human Erythrocute Separation

The column was fitted with two electrodes at the supply and collection end of the column. The column was then filled with isotonic buffered saline solution [5] with a conductivity of 17,500 μmhos/cm. The sheep and human erythrocytes were prepared from EDTA-treated, fresh blood by washing three times, repeating centrifugation, and decanting the supernatant. The final erythrocyte suspensions were adjusted to a hematocrit of 40. Equal volumes of human and sheep erythrocyte suspensions were combined.

The sample mixture (0.3 ml) was injected into the sample port. The column was eluted (11.1 ml/hr) with buffered saline solution while the column was rotated around the major axis. Minor axis column rotation was set at 5 rpm cc. Three runs were made with a 0 voltage gradient, +2000 V collection gradient, and -2000 V collection gradient.

The column outlet was continuously monitored with a Uvicord III detector set at a wavelength of 280 nm and fractions were collected in test tubes using an LKB 17000 fraction collector. All fractions were analyzed using an improved Neubauer hemacytometer.

The column resistance was measured to be 21.7 megaohms when filled with the isotonic buffered saline. The current flow measured was 93 μA at 2000 V.

Cell mobilities were measured with a Rank Microelectrophoresis System. The measured mobilities of fresh sheep and human erythrocytes in buffered, isotonic saline were -1.00 ± 0.02 and -0.93 ± 0.02, respectively.

IV. RESULTS

A. Single Phase Fresh Sheep/Human Erythrocyte Separation

An inspection of the absorption curves presented in Figure 8 reveals three major fractions. The first small group of peaks represent hemoglobin from hemolysis of erythrocytes. The second and third peak represent sheep and human erythrocyte fractions, respectively.

Further analyais of cell counts of all fractions collected reveals sub-populations of sheep and human erythrocytes (Fig. 9). Cell population analysis also shows the overlap of these subpopulations.

B. Single Phase Fixed Sheep/Human Erythrocyte Separation

An improved separation was expected over the fresh erythrocyte separation due to increased rigidity of cell membranes caused by the fixing procedure. The almost 3:1 volume difference of human and sheep erythrocytes should have given a good separation.

Absorption and cell distribution data shown in Figures 10 and 11 indicate that no separation of cells occurred. The experiment was repeated using different combinations of rotational directions and flow rates. Each attempt presented similar results. The inability to separate fixed erythrocytes could be due to differences in fixed cell walls and the cell interaction among themselves and the column wall.

C. Single Phase Electrophoretically Enhanced Fresh Sheep/Human Erythrocyte Separation

At a potential of 2000 V applied across the column, one realizes a field strength of 1.053 V/cm. Nominal sheep and human retention times are 95 and 140 min, respectively. Despite the long retention times one expects the sheep and human erythrocytes to move 0.6 cm and 0.8 cm, respectively, further along the column than they would without the 2000 V potential. This additional movement is clearly negligible given the 1900 cm column length.

Figures 12, 13, and 14 show the appreciable effects of the applied voltage gradient. From the figures one sees that there is a 2-tube separation between peaks of the 0 V baseline run, 0 tube separation for a +2000 V collection end gradient and a 5-tube separation for the -2000 V collection end gradient.

At present no definite explanation can be given for this effect. One possibility might be a charge related interaction between cells and the column surface. Another factor present during the electrophoretically enhanced separation would be electroosmosis. It is, however, unlikely that electroosmosis would present an appreciable effect considering the small field strength and large length of the column.

V. DISCUSSION

Separating different types of biological materials with the rotating coil planet centrifuge is conceptionally very appealing since relatively mild mechanical agitation and cell favoring buffers are used. Many separations were attempted in which the various operating parameters were adjusted to produce the best possible separation of model particles. The quality of these separations, unfortunately, was less than expected.

One inherent problem with the rotating coil planet centrifuge is the fact that even a small sample (0.2 cc) is distributed along a column length of approximately 25 cm after injection into the sample port. All runs produced a certain amount of hemolysis depending upon cell age. Occasionally cells would become lodged after having moved through a large length of the column.

Experiments were performed back to back using the same samples and identical operating parameters in order to check the reproducibility. Retention times varied as much as ±5 percent of the total retention times. Often, however, no separation of cells occurred in a run in which conditions were identical to a run in which separation occurred. In the cases where no separation occurred, no apparent cause, such as blockage, could be detected.

The rotating coil planet centrifuge offers viable cells a benign environment in which to be separated. The quality and reproducibility of single phase separation, utilizing the coil planet centrifuge, requires improvement. Such improvement is likely to occur with an improved understanding of the basic mechanisms by which particles are separated in the coil planet centrifuge.

The present analysis suggests that substantial improvement may be attained with the exploitation of the electrophoretic effects presented.

The axial movement of particles within the column was described by Ito [1,5,6] as being caused by an Archemedian screw force. The separation principle as described by Ito neglects as a first approximation any lateral movement of particles within the column. A paper which predicts particle retention times as a function of axial laminar flow velocities is presently in preparation by the author. Hopefully, a better understanding of the rotating coil centrifuge separation mechanism will be attained through the use of this field flow fractionation model.

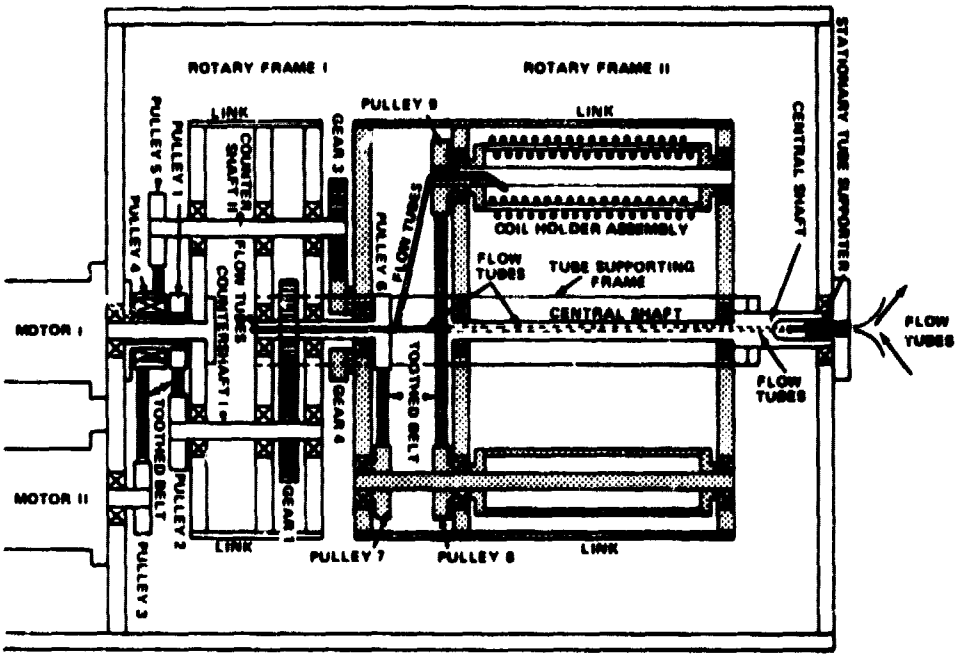


Figure 1. Cross sectional view of the coil planet centrifuge.

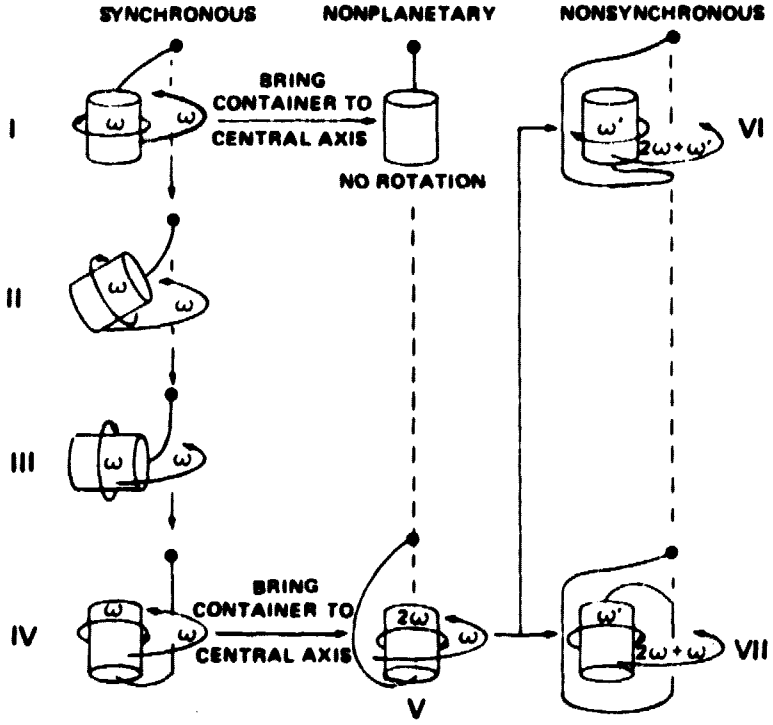


Figure 2. Coil planet centrifugation schemes.

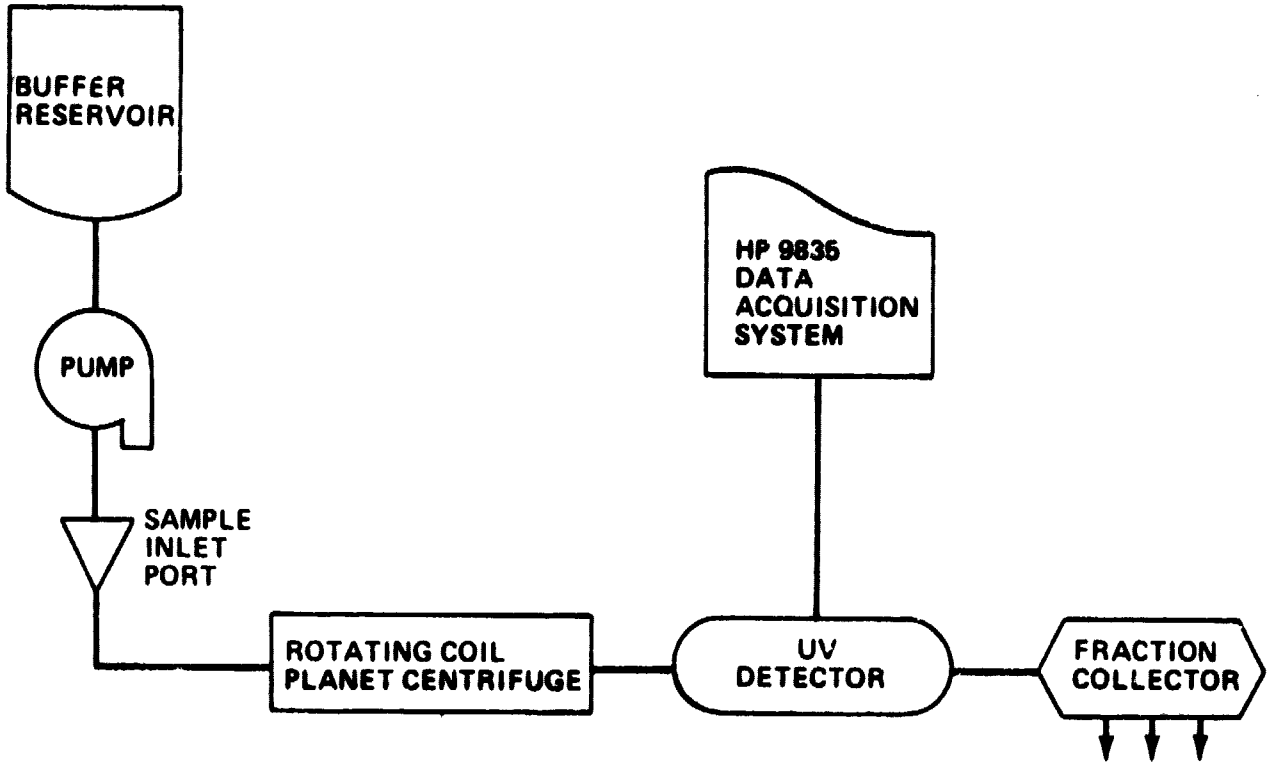
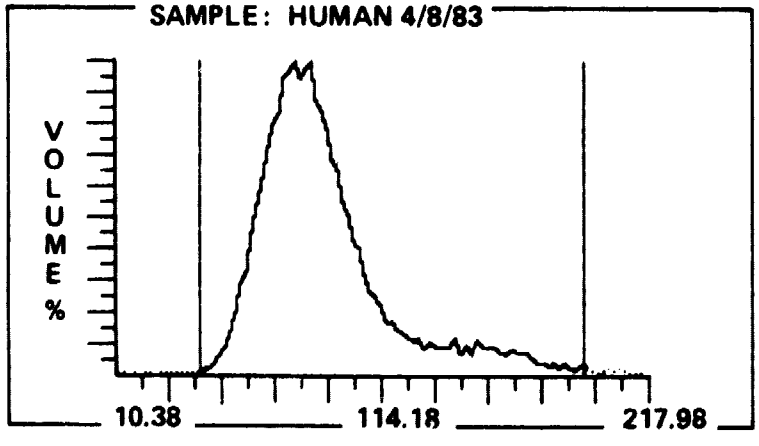


Figure 3. Rotating coil planet centrifuge system functional diagram.

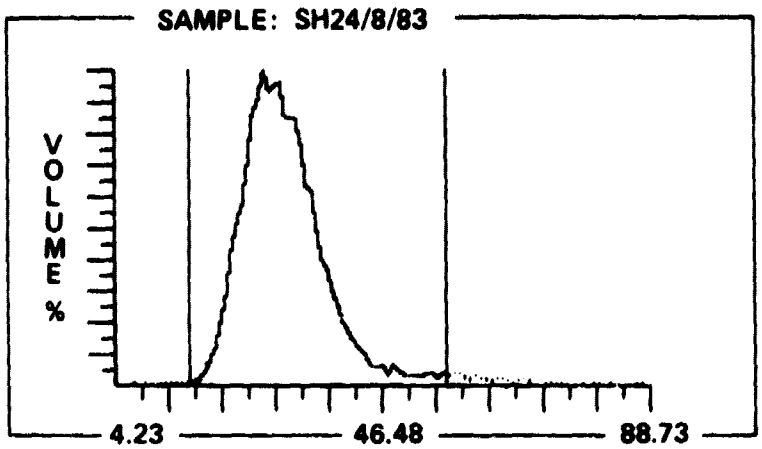


VOLUME STATISTICS

SAMPLE:	HUMAN 4/8/83
OPERATOR:	FRED HERRMANN
DATE:	04/08/83
ELECTROLYTE:	ISOTON II
APERTURE:	50 MICROMETERS
THRESHOLD FACTOR:	2.07601678FL*
SHAPE FACTOR:	.4904
BCT: 5 WW: 100	
SAMPLE:	HUMAN 4/8/83
CALCULATIONS BETWEEN: 43.6 & 196.15 FL	
SAMPLE:	HUMAN 4/8/83
CALCULATIONS BETWEEN: 43.6 & 196.15 FL	
TOTAL VOLUME:	1683037.18
MEAN:	93.66 FL
MEDIAN:	87.76 FL
MEDIAN/MEAN RATIO:	.94
STD. DEVIATION:	26.19 FL

* FL = 1 um^3

Figure 4. Fresh human erythrocyte size distribution.

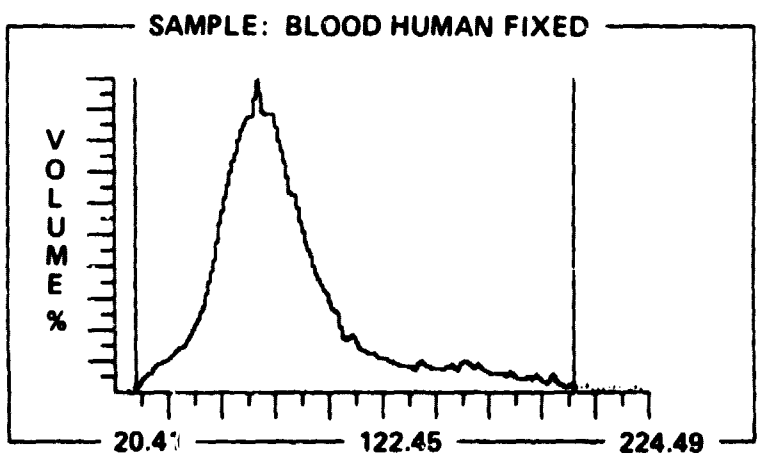


SAMPLE:	SH2 4/8/83
OPERATOR:	FRED HERRMAN
DATE:	04/08/83
ELECTROLYTE:	ISOTON II
APERTURE:	50 MICROMETRES
THRESHOLD FACTOR:	.845018779FL*
SHAPE FACTOR:	.8024
BCT: 5 WW: 100	

SAMPLE:	SH2 4/8/83
CALCULATIONS BETWEEN: 16.06 & 57.46 FL	
TOTAL VOLUME:	466314.79
MEAN:	31.53 FL
MEDIAN:	30.76 FL
MEDIAN/MEAN RATIO:	.98
STD. DEVIATION:	6.32

*FL = 1um^3

Figure 5. Fresh sheep erythrocyte size distribution.

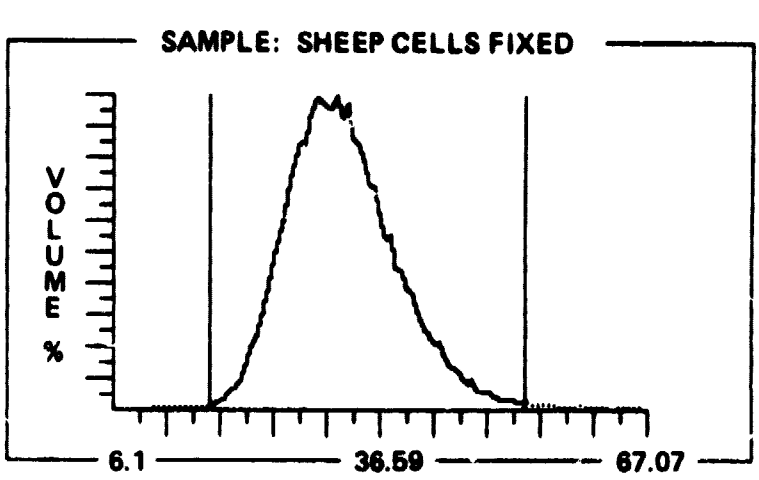


SAMPLE:	BLOOD HUMAN FIXED
OPERATOR:	FRED HERRMAN
DATE:	04/21/83
ELECTROLYTE:	ISOTON II
APERTURE:	50 MICROMETRES
THRESHOLD FACTOR:	4.08163265FL*
SHAPE FACTOR:	.49
BCT: 5 WW: 100	

SAMPLE:	BLOOD HUMAN FIXED
CALCULATIONS BETWEEN: 28.57 & 197.96 FL	
TOTAL VOLUME:	1551124.49
MEAN:	87.08 FL
MEDIAN:	80.83 FL
MEDIAN/MEAN RATIO:	.93
STD. DEVIATION:	29.23 FL

*FL = 1 um^3

Figure 6. Fixed human erythrocyte size distribution.



SAMPLE:	SHEEP CELLS FIXED
OPERATOR:	FRED HERRMANN
DATE:	04/21/83
ELECTROLYTE:	ISOTON II
APERTURE:	50 MICROMETRES
THRESHOLD FACTOR:	1.2186122FL*
SHAPE FACTOR:	.41
BCT: 5 WW: 50	

SAMPLE:	SHEEP CELLS FIXED
CALCULATIONS BETWEEN: 17.07 & 53.06 FL	
TOTAL VOLUME:	700267.06
MEAN:	32.58 FL
MEDIAN:	32.04 FL
MEDIAN/MEAN RATIO:	.98
STD. DEVIATION:	5.98 FL

*FL = 1 um^3

Figure 7. Fixed sheep erythrocyte size distribution.

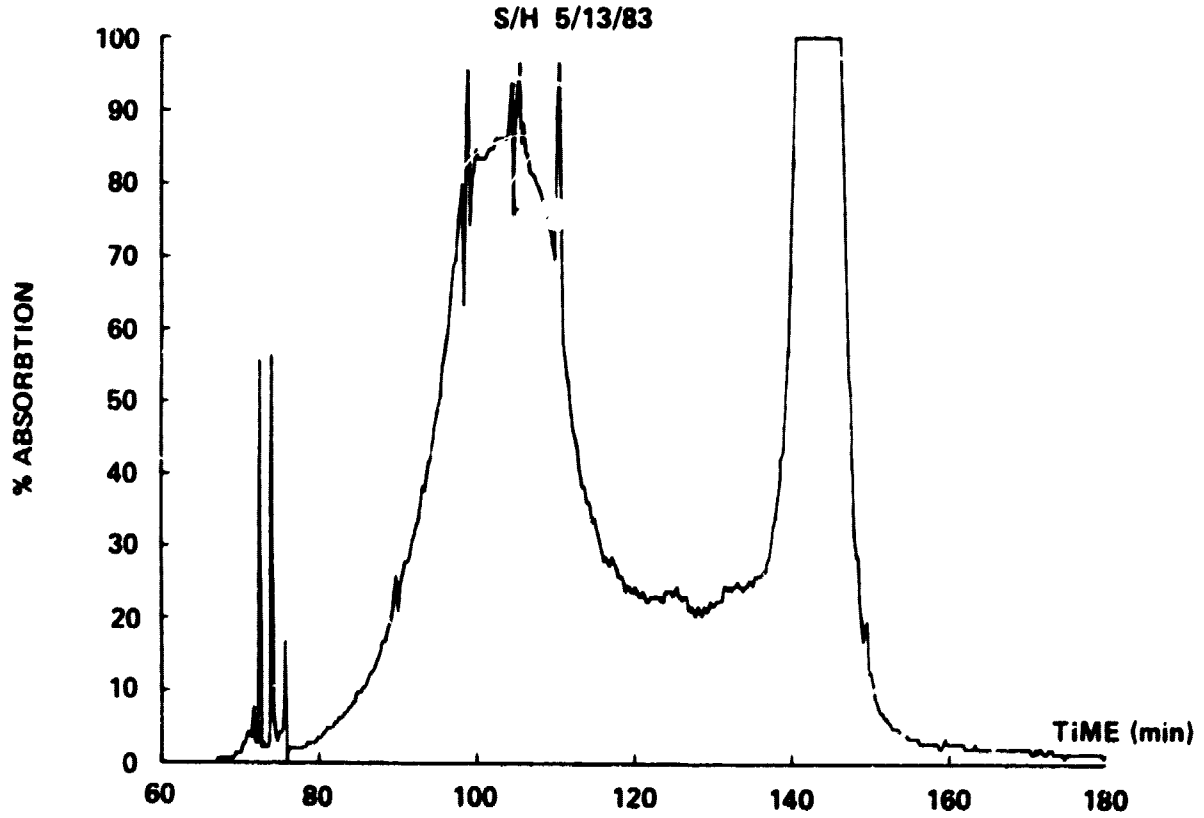


Figure 8. Fresh erythrocyte single phase separation absorption curve.

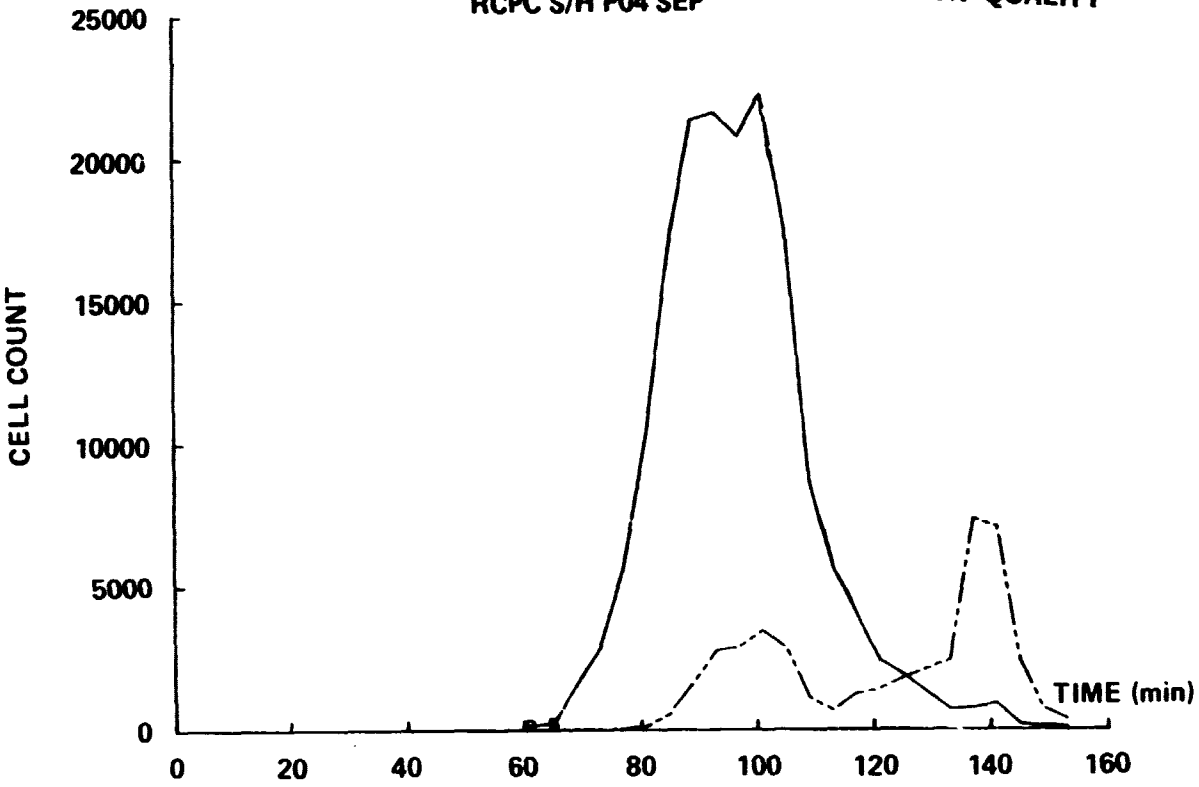


Figure 9. Fresh erythrocyte single phase population distribution.

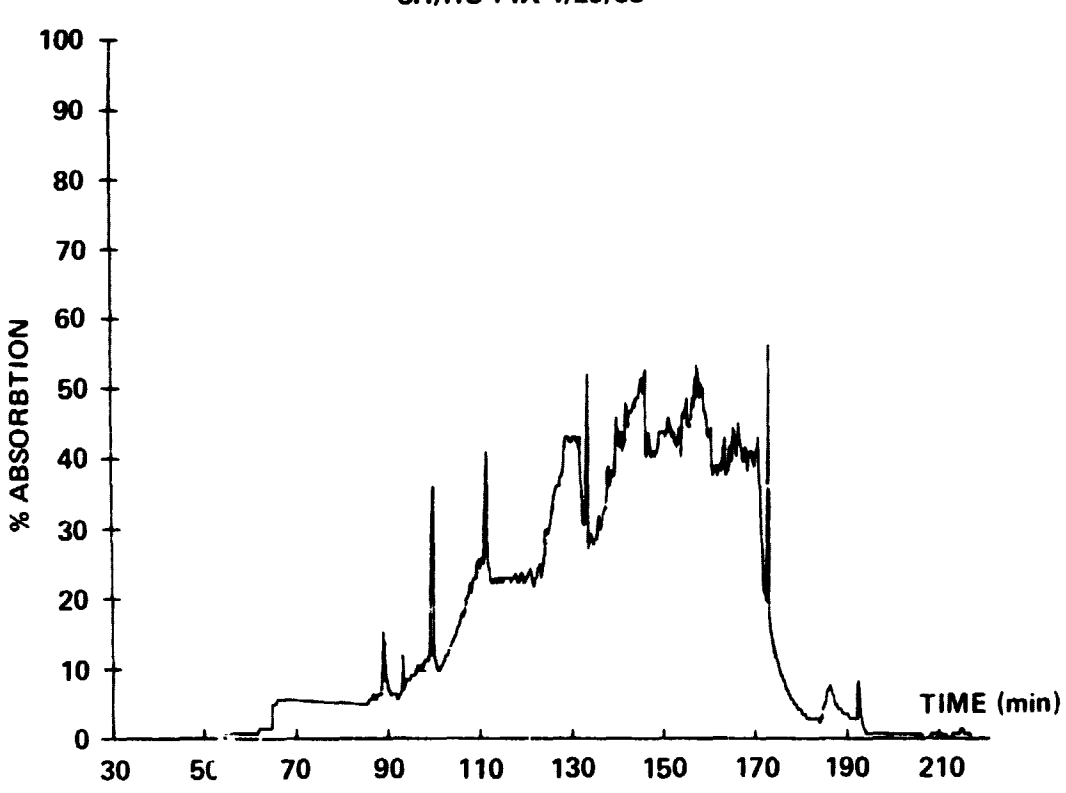


Figure 10. Fixed erythrocyte single phase separation absorption curve.

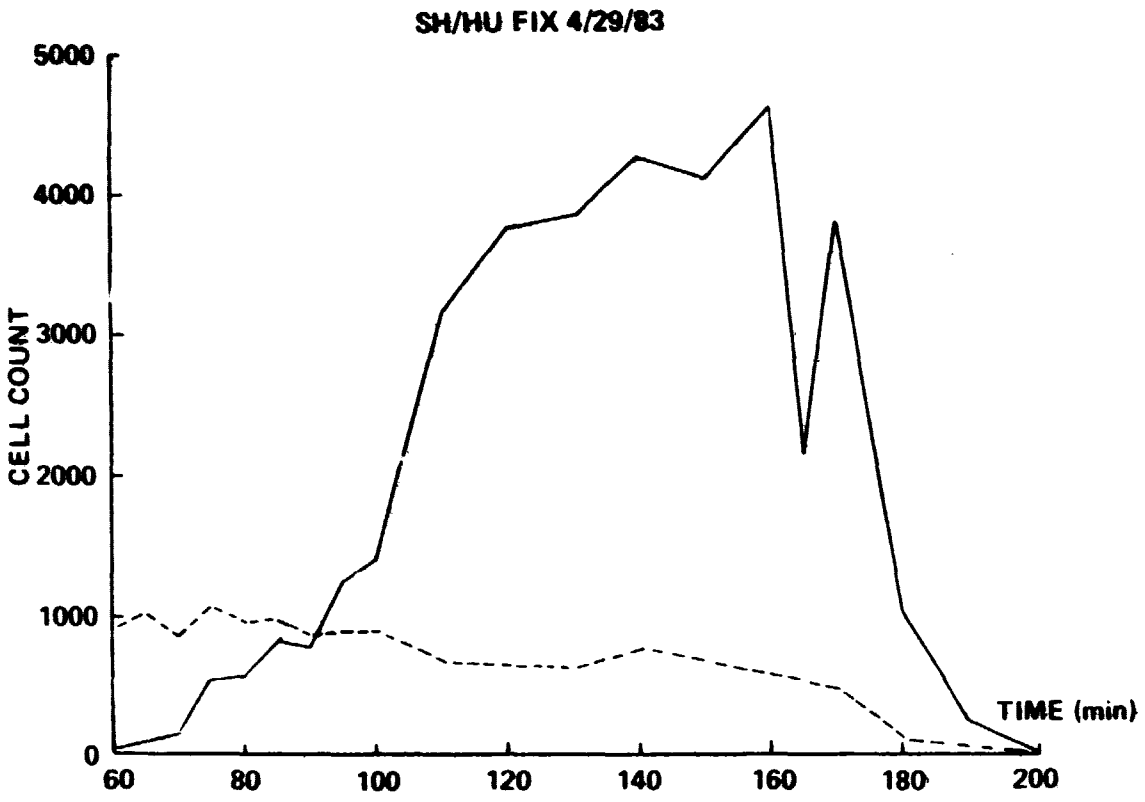


Figure 11. Fixed erythrocyte single phase population distribution.

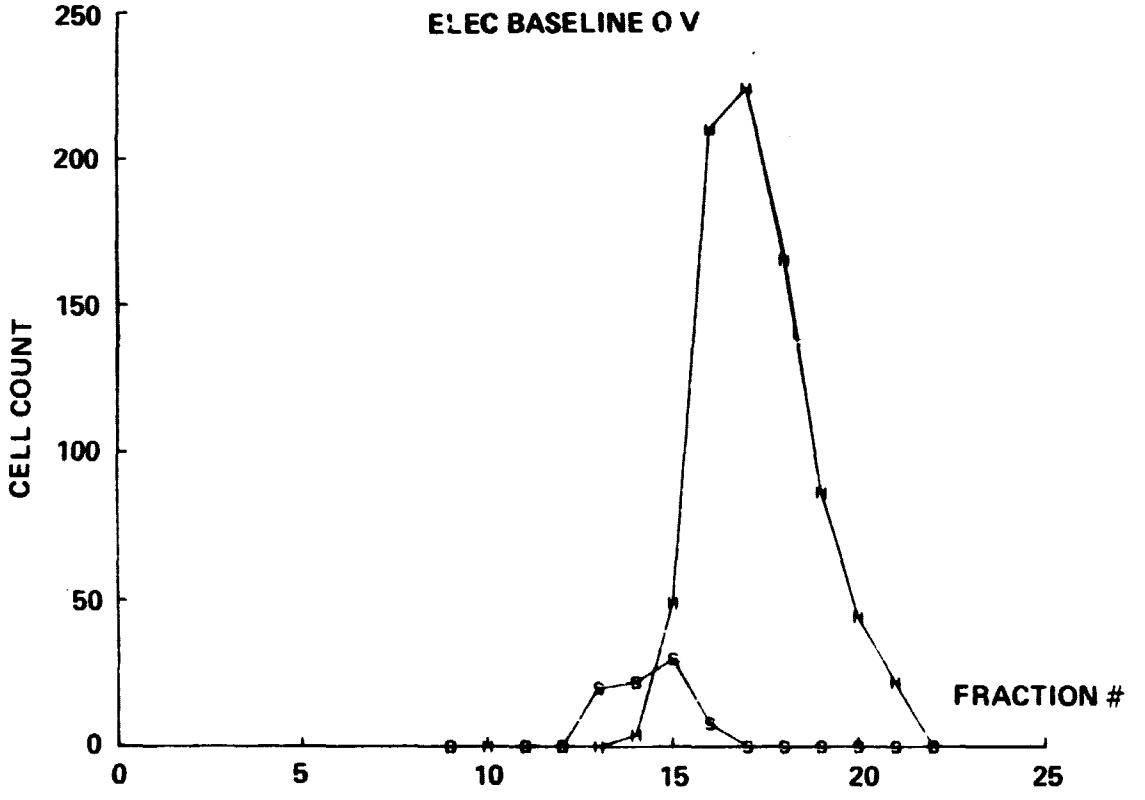


Figure 12. Fresh erythrocyte electrically enhanced 0 V baseline population distribution.

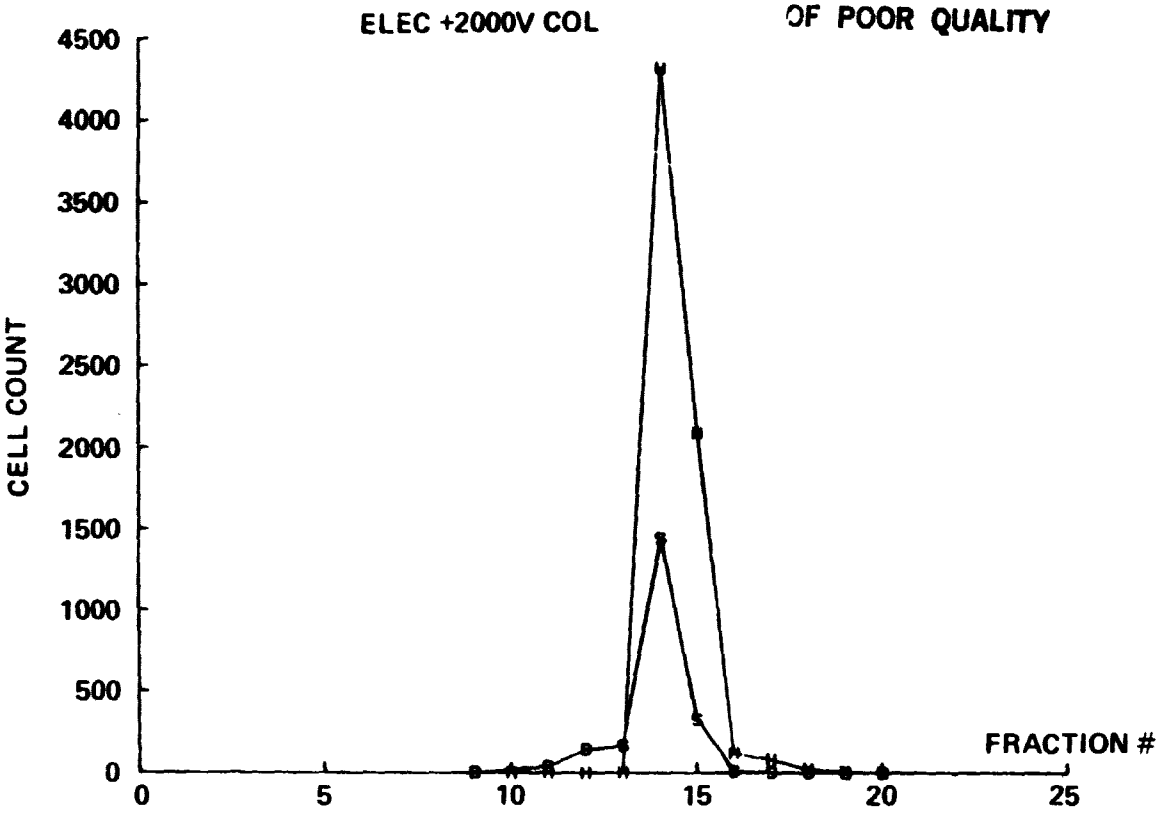


Figure 13. +2000 V (collection end) enhanced population distribution.

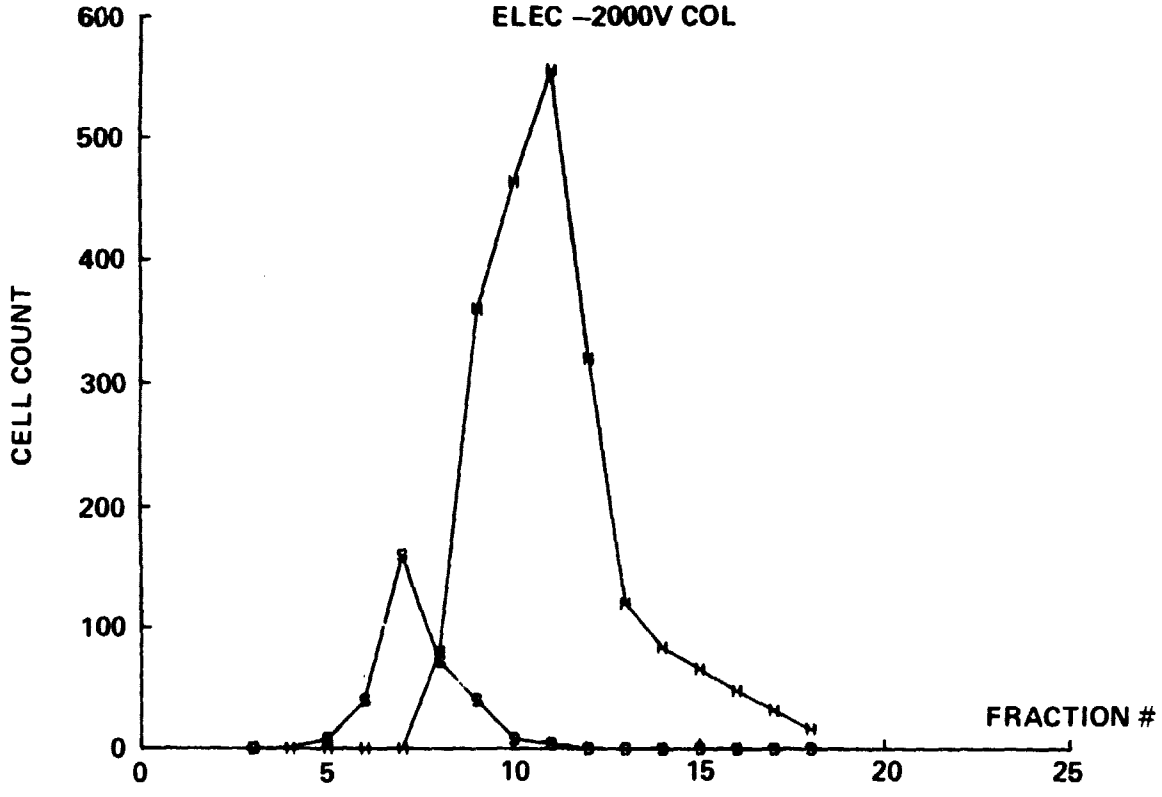


Figure 14. -2000 V (collection end) enhanced population distribution.

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APPROVAL

COIL PLANET CENTRIFUGATION AS A MEANS FOR SMALL PARTICLE SEPARATION

By Frederick T. Herrmann

The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

A. J. DESSLER
Director, Space Science Laboratory