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

Stability of Erythrocyte Suspensions Layered on Stationary and Flowing Liquids

S. N. Omenyi[ab]; P. H. Rhodes[a]; R. S. Snyder[a]
[a] LABORATORY NASA/GEORGE C. MARSHALL SPACE FLIGHT CENTER, SEPARATION PROCESSES BRANCH SPACE SCIENCES, ALABAMA [b] Universities Space Research Association Visiting Scientist.,

To cite this Article Omenyi, S. N. , Rhodes, P. H. and Snyder, R. S.(1982) 'Stability of Erythrocyte Suspensions Layered on Stationary and Flowing Liquids', Separation Science and Technology, 17: 6, 849 — 857
To link to this Article: DOI: 10.1080/01496398208058326
URL: http://dx.doi.org/10.1080/01496398208058326

SEPARATION SCIENCE AND TECHNOLOGY, 17(6), pp. 849–857, 1982

Stability of Erythrocyte Suspensions Layered on Stationary and Flowing Liquids

S. N. OMENYI,* P. H. RHODES, and R. S. SNYDER†

SEPARATION PROCESSES BRANCH
SPACE SCIENCES LABORATORY
NASA/GEORGE C. MARSHALL SPACE FLIGHT CENTER
ALABAMA 35812

Abstract

The apparent stability of erythrocyte suspensions layered on stationary and flowing Ficoll solutions was studied considering the effects of particle concentration, type and size, and the different flow rates of the particle suspensions and chamber liquid. The data from the flowing system were empirically fitted and, when extrapolated to zero chamber liquid flow rate, gave values comparable to the data from the stationary system, thus confirming the validity of the data and our approach to obtain that data.

INTRODUCTION

The fractionation of a biological cell mixture by centrifugation or zone electrophoresis often requires inserting the dilute particle suspension as a thin layer or narrow stream on a solution of nonconducting gradient forming compound, e.g., sucrose or Ficoll. Under an applied field, e.g., gravitational or electrical, the particles in the zone migrate to form different layers according to their size and/or density, if the external field is gravitational, or according to their electrophoretic mobility, if the field is electrical.

It has been observed that the suspension layer can become unstable even though it is initially less dense than the supporting liquid below. This is usually due to a density inversion at the interface between the suspension layer and the supporting liquid which consequently results in excessive zonal

*Universities Space Research Association Visiting Scientist.

†To whom all correspondence should be directed.

spreading by convection. The above phenomenon, generally referred to as "streaming" or "droplet sedimentation" (*1–5*), depends on the particle density, concentration and surface properties, composition and density of the supporting liquid gradient, and mass diffusion rates of particles and solutes in liquid cushion. Hitherto, the above factors have been studied in stationary containers using small particles such as viruses, proteins, or blue dextran (*2*). In order to gain further insight into the initiation of the droplet sedimentation phenomenon, the behavior of erythrocytes, as model particles, was observed under stationary and flowing conditions.

As an indication of instability in the stationary system, the droplet formation time, or the time interval which occurs between layering of the suspension and the appearance of droplets carrying trapped particles at the interface, was measured. In the flowing system the flow rate of the chamber liquid and sample at which sample suspension stream begins to broaden or sediment was determined. If the sample stream did not vary significantly with time, it was considered to be stable.

MATERIALS

The particle materials used were glutaraldehyde-fixed (*7*) erythrocytes from turkey, chicken, man, pig, dog, and horse, which are well characterized and have the necessary variety of size and charge. The densities and diameters are listed in Table 1*a*. Each particle species was suspended in unbuffered 0.15 *M* NaCl aqueous solution. The supporting liquid was 2% (w/v) Ficoll (Molecular weight = 400,000; Pharmacia Fine Chemicals, Piscataway, New Jersey) in 0.15 *M* NaCl aqueous solution. The densities of 2% Ficoll solution and 0.15 *M* NaCl solution determined by pycnometry were 1.009 and 1.002 g/mL, respectively, at 25°C.

METHODS

The suspension of a given particle species of known concentration, N cells/mL, was carefully layered on a stationary 2% Ficoll solution contained in a thermostated rectangular Plexiglas chamber as described in Ref. 6. In each case the droplet formation time was recorded for various suspension concentrations and for different particle species and plotted in Fig. 1. In the flowing system the erythrocyte suspension was inserted at a known rate, M_p mL/h, with a syringe pump into the horizontal interface between two liquid layers flowing at M_s mL/min, e.g., physiological saline alone as the upper layer and with 2% Ficoll solution as the lower layer, contained in a STAFLO-type (*8*) Plexiglas migration chamber. In each case the vertical

TABLE 1

Physical Properties of the Particles and the Experimental Data

Material	Diameter (μm)	Density (g/mL)	N_0 (cells/mL)	ϕ_f ($n = \frac{1}{2}$)	M_s (mL/min)	M_p (mL/h)	M_s/M_p
			(a) Stationary System				
Turkey	11.3	1.096					
Chicken	10.6	1.095	1.1×10^8	21.94			
Human	7.5	1.093	1.8×10^8	12.45			
Pig	7.4	1.091	2.7×10^8	17.54			
Dog	7.0	1.090	2.6×10^8	14.14			
Horse	5.5	1.091	4.5×10^8	12.00			
			(b) Flowing System				
Turkey			5.41×10^8	132.18	27.40	0.8	2055
					20.55	0.6	2055
			3.45×10^8	84.31	8.16	0.2	1319
					23.08	7.3	
			2.24×10^8	54.73	27.40	3.0	548
Chicken			3.45×10^8	68.87	23.08	1.2	1154
			2.30×10^8	45.89	15.80	3.1	306
Pig			3.45×10^8	22.42	8.16	5.1	96
Horse			3.45×10^8	9.12	8.16	9.2	53
					23.08	12.5	111

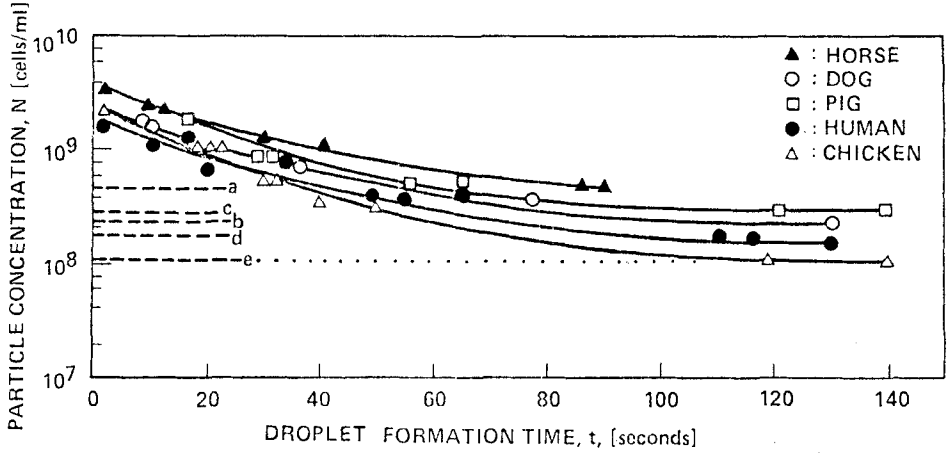

FIG. 1. Variation of droplet formation times with suspension concentration. The broken lines represent the critical concentrations for droplet formation and are given as (a) horse, 4.5×10^8; (b) dog, 2.6×10^8; (c) pig, 2.7×10^8; (d) human, 1.8×10^8; and (e) chicken, 1.1×10^8 cells/mL.

deflection of sample stream was measured at a fixed distance in the chamber, to within ±0.1 mm.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between the suspension concentration and the corresponding droplet formation time for various materials in a stationary system. It is clear that the droplet formation time decreased as the particle suspension became more concentrated. The lower limit of each curve, i.e., where no droplets are observed, corresponds to the critical particle concentration, N_0, for droplet formation. These values of N_0, listed in Table 1*a*, show clearly that N_0 and hence suspension stability depends on material type and size. Least stability occurs for the large turkey cells whereas the small horse cells are most stable.

In the flowing system, stability is favored at low sample flow rates (M_p) (see Figs. 2 to 4) and at high chamber liquid flow rates (M_s) (see Figs. 3 and 4) for a given particle material. In the case of stationary systems, stability can be explained in terms of droplet formation times. In the flowing system, if the time a given particle suspension remained on the liquid cushion was less than the time required for the formation of droplets of particles, then the particle suspension would remain stable. This means, therefore, that an increase in chamber liquid flow rate which reduces the residence time of a given particle on the liquid cushion will enhance the apparent stability of particle suspension.

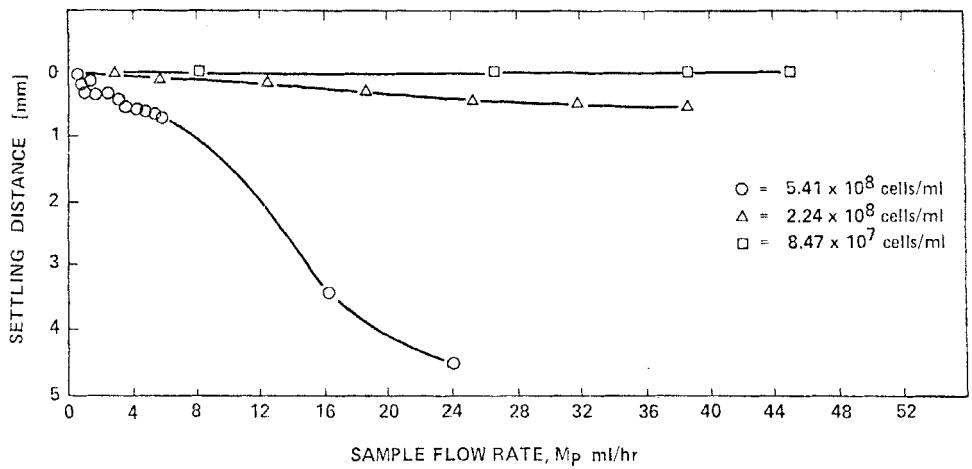

FIG. 2. Concentration dependence of the settling of turkey red blood cells at constant chamber liquid flow rate, $M_s = 27.4$ mL/min, and for various sample flow rates, M_p.

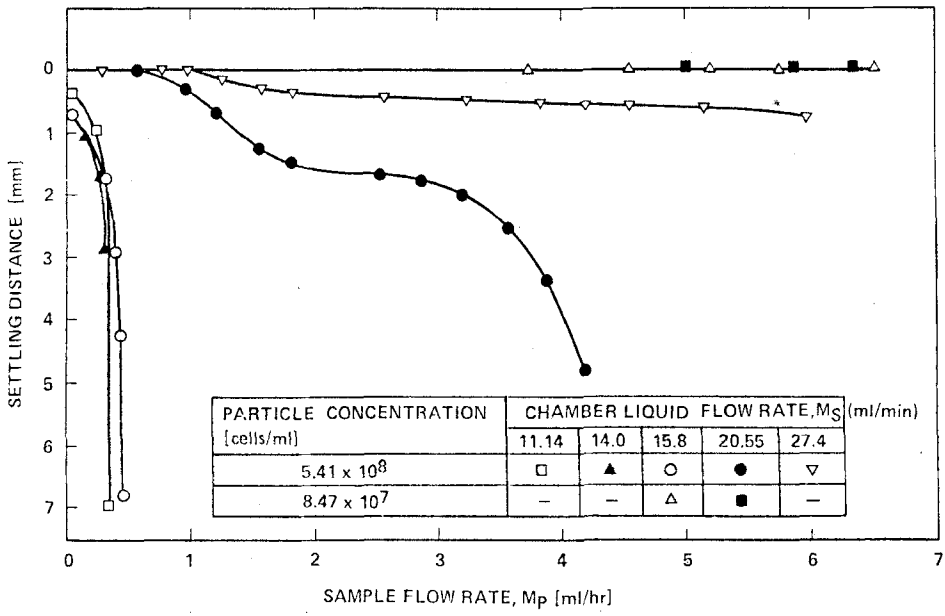

FIG. 3. Dependence of the settling of turkey cells on chamber liquid and sample flow rates.

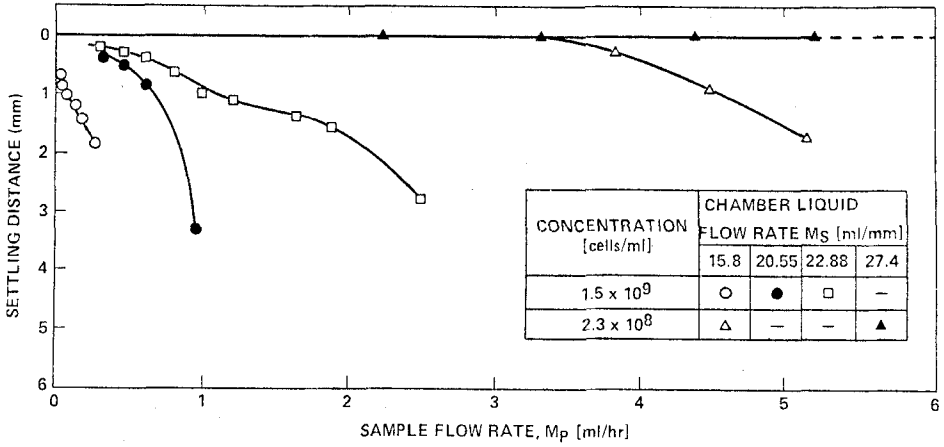

FIG. 4. Dependence of the settling of chicken cells on chamber liquid and sample flow rates.

The apparent stability of suspensions layered on a flowing liquid cushion also depends on particle type as can be seen in Figs. 5 and 6 which give a different curve for each particle species (except chicken and turkey cells in Fig. 5). The size dependence observed in the stationary system does not appear to hold in the flowing system. Pig and human cells are almost of the same size; yet in Fig. 5, pig cells are more stable. In Fig. 6, small horse cells are less stable than the larger pig and human cells, while the small chicken cells are less stable than the large turkey cells. The apparent stability of these cells must therefore also depend on the hydrodynamic disturbances associated with a flowing liquid and on the balance between the attractive van der Waals forces and the double-layer repulsion (see Ref. *6*). Apparently, fluid mechanical differences and surface properties can be more significant than size effects.

The major difference between the stationary and flowing systems is in the existence of flow rates M_s and M_p in the latter system. M_s and M_p for each particle species are listed in Table 1*b*. We have shown that the apparent sample stream instability increased with increasing M_p, but decreased with increasing M_s. Thus apparent suspension stability should depend on the ratio M_s/M_p. We can therefore write the condition for the onset of sample stream instability as a function ϕ_f that is greater than or equal to a constant C plus a certain function of M_s/M_p, i.e.,

$$\phi_f \geq C + f\left(\frac{M_s}{M_p}\right), \qquad M_p \neq 0 \tag{1}$$

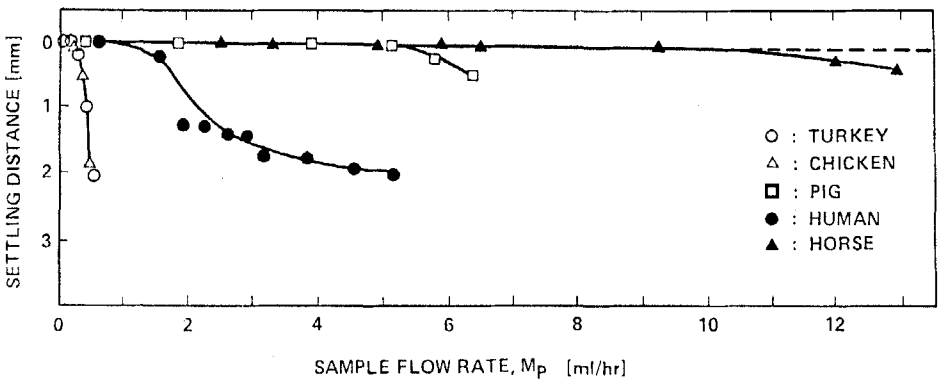

FIG. 5. Apparent stability of sample streams at constant chamber liquid flow rate $M_s = 8.16$ mL/min for different particulate materials at constant particle concentration $N = 3.45 \times 10^8$ cells/mL.

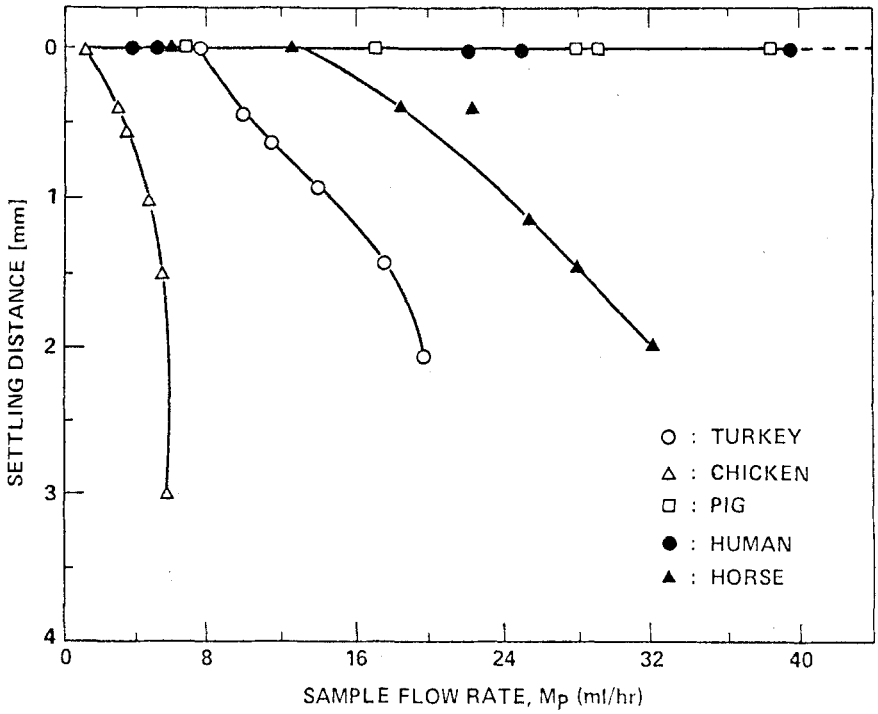

FIG. 6. Apparent stability of sample streams at constant chamber liquid flow rate $M_s = 23.08$ mL/min for different particulate materials at constant particle concentration $N = 3.45 \times 10^8$ cells/mL.

where (*3–5*)

$$\phi_f = \frac{4\pi}{3} a_p^3 N_0 \left(\frac{\rho_p - \rho_L}{\rho_s - \rho_L} \right) \left(\frac{D_s}{D_p} \right)^n \tag{2}$$

and n has values ranging from $\frac{1}{2}$ (*4*) to $\frac{3}{2}$ (*5*). D is the diffusion coefficient, ρ is the density, a is the particle radius, and the subscripts p, L, and s represent the particle, the upper liquid layer, and the supporting liquid cushion, respectively. For a stationary system, $M_s = 0$ and hence $M_s/M_p = 0$.

A problem with the use of Eq. (2) is that n is not known exactly and D_p for erythrocytes is very low. To correlate our data with Eq. (1), we assume n

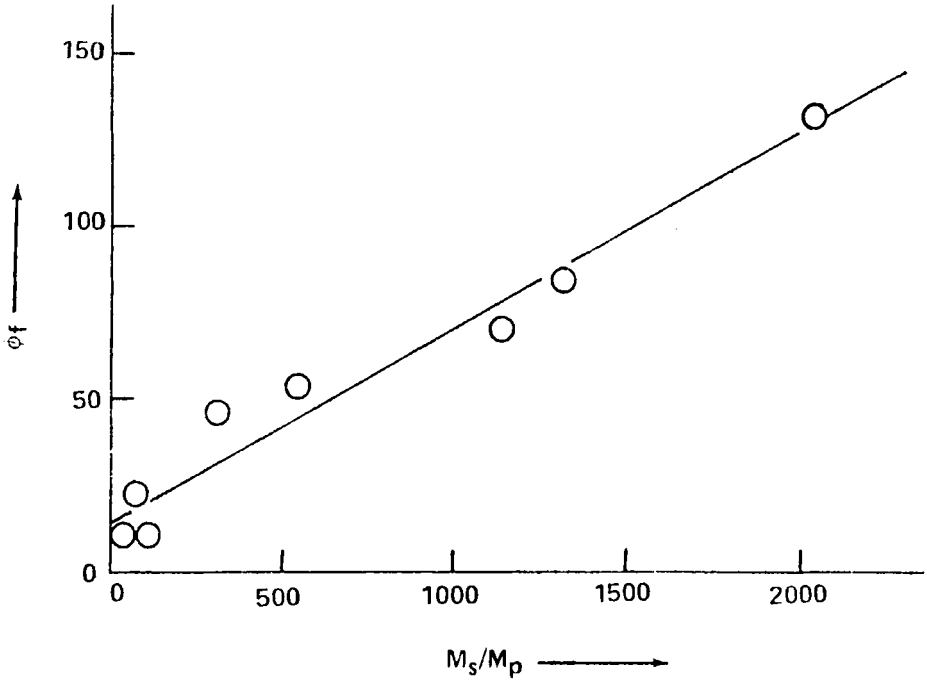

FIG. 7. Correlation of the experimental data for the flowing systems. The equation of the solid line, obtained by linear regression analysis, is $\phi_f = 13.51 + 0.057\,(M_s/M_p)$, with a correlation coefficient of 0.97.

$= \frac{1}{2}$, $D_p \approx 5.3 \times 10^{-10}$ cm^2/s for all cells (estimated from the Stokes-Einstein relation), and $D_s \approx 2.97 \times 10^{-7}$ cm^2/s. Equation (1) was therefore correlated by linear regression analysis (see Fig. 7) with the data of the flowing system given in Table 1*b* as

$$\phi_f \geq 13.51 + 0.057 \frac{M_s}{M_p}, \qquad M_p \neq 0 \tag{3}$$

with a correlation coefficient of 0.97. ϕ_f, calculated with the data of Table 1*a* (for the stationary system) in conjunction with Eq. (2) and listed also in Table 1*a*, varied from 12.0 to 21.9. The scatter in these values could be due to the differences in surface properties of the particles which are not incorporated in Eq. (2). If Eq. (3) (for the flowing system) is extrapolated to zero chamber liquid flow rate, i.e., $M_s = 0$, we obtain $\phi_f \geq 13.51$ which is in the range of values reported in Table 1*a* for stationary systems. The close

agreement between these values confirms the validity of our approach and of the data.

Acknowledgments

This work was accomplished while one of us (S.N.O.) held an NRC Resident Research Associateship. We thank Dr C. J. van Oss, of the State University of New York at Buffalo, for his valuable suggestions.

REFERENCES

1. M. K. Brakke, *Arch. Biochem. Biophys., 55,* 175 (1955).
2. P. Nason, V. Schumaker, B. Halsall, and J. Schevedes, *Biopolymers, 7,* 241 (1969).
3. J. A. Meuwissen and K. P. Heirwegh, *Biochem. Biophys. Res. Commun., 41,* 675 (1970).
4. H. Svensson, L. Hagdahl, and K. D. Lerner, *Sci. Tools, 4,* 1 (1957).
5. W. K. Sartory, *Biopolymers, 7,* 251 (1969).
6. S. N. Omenyi, R. S. Snyder, C. J. van Oss, D. R. Absolom, and A. W. Neumann, *J. Colloid Interface Sci., 81,* 402 (1981).
7. P. S. Vassar, J. M. Hards, D. E. Brooks, B. Hadenberger, and G. V. F. Seaman, *J. Cell Biol., 53,* 809 (1972).
8. H. C. Mel, *J. Chem. Phys., 31,* 559 (1959).

Received by editor August 3, 1981