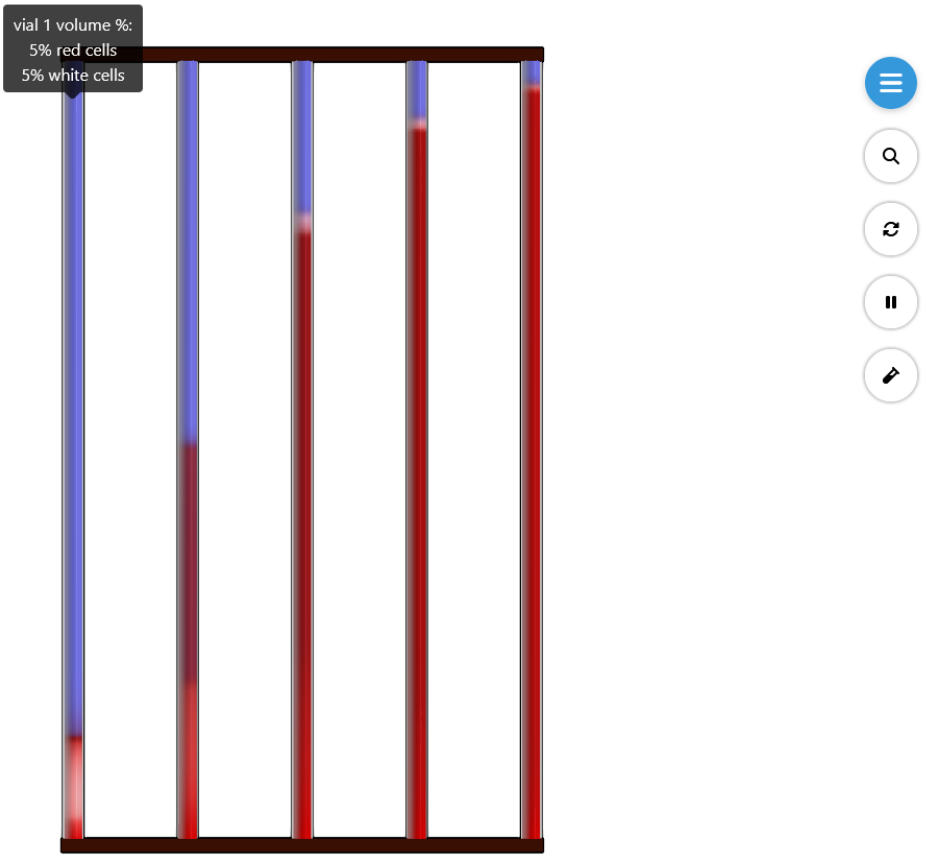
Blood Cell Separations Worksheet

Name(s): _____

Student Learning Objectives:

1. Understand the effect of particle diameter on drag force as particles settle through fluid.
2. Calculate effective porosity and relate to particle collisions in a dense suspension and how they hinder particle sedimentation.
3. Understand how particle concentration alters the density of the suspension and particle settling velocity.
4. Describe the different sedimentation scenarios and parameters that dominate particle settling.
5. Understand how gravitational force versus centrifugal force affects sedimentation.
6. Relate objectives 1 through 5 to blood cell separations and biomedical applications.

Equipment



To easily observe trends, this simulation used beads 50 times the size of white blood cells (WBCs) and red blood cells (RBCs). For faster settling in this digital experiment, the density of the suspension is smaller than the 1.026 g/cm^3 for blood plasma. The column height is 305 mm, and its inner diameter is 7 mm, but the simulation exaggerates the column diameter so the cell behavior can be more easily visualized.

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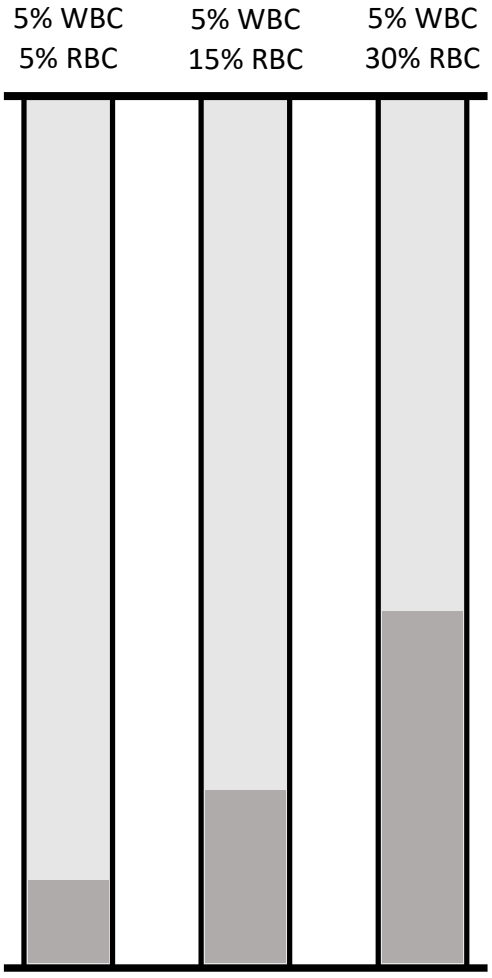
Bead Information	Color	Diameter, d [μm]	Density, ρ [g/cc]
Red blood cell (RBC)	Red	275	1.08
White blood cell (WBC)	White	550	1.00

Suspending solution: 70% EtOH/30% H_2O; density, ρ = 0.868 g/cc; viscosity, μ = 2.15 cP

Before starting the experiment

On the figure below, which shows only 3 vials, mark dots or circles with a pen to predict how the WBCs will settle in the column with respect to increasing concentration of RBCs (dark grey shaded area). *Hint: will the WBCs settle at the bottom, spread throughout the suspension, or settle at the top?*



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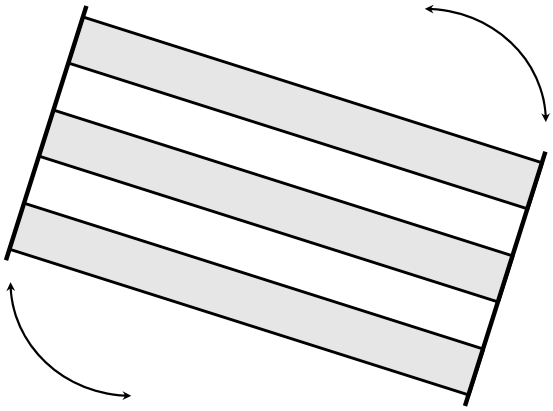
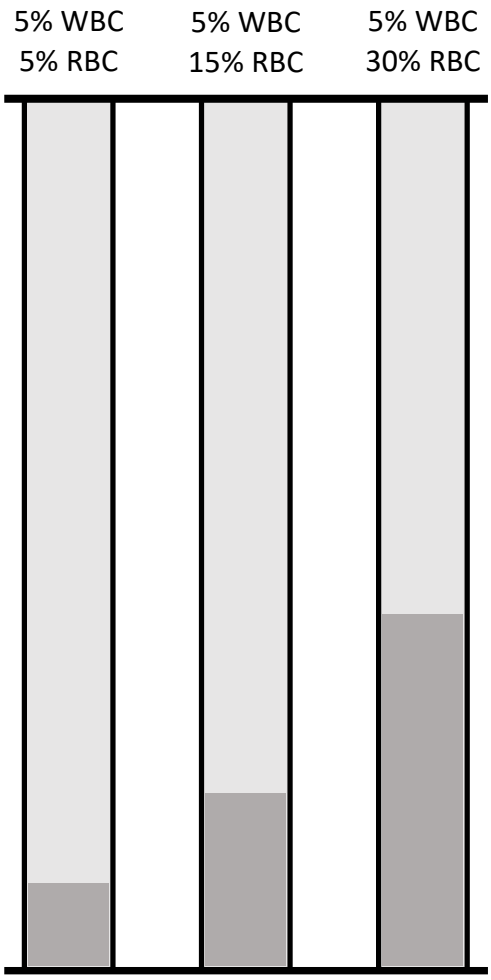
Experiment:

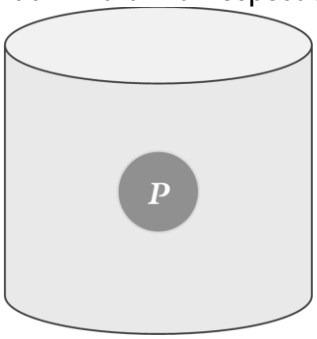
1. Click on the two arrows in a circle to invert the module back and forth to mix the beads.
2. Closely watch how the white beads settle and interact with the red beads across the five columns.
3. Mark dots or circles using a pen to show how the white beads settled within the red beds (grey shaded area) on the schematic in the following page.





Write your observations on how the white beads settled with respect to increasing concentration of the red beads in the three columns shown on the left:

Forces Acting on Particles Settling in Suspension:
On the diagram below, draw the forces and their orientation (up/down arrows) acting on a single particle as it settles through a column downward with respect to gravity.



4. To better see where red and white beads are located, click on the magnifying glass and move it over the columns.

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Stokes' Law and Terminal Velocity:

The forces acting on a single particle of diameter "d" settling in suspension are drag force "F_D," buoyant force, "F_B," and gravitational force "F_G."

$$F_D = 3\pi\mu d v_t$$

$$F_B = \frac{1}{6}\pi d^3 \rho_f g$$

$$F_G = \frac{1}{6}\pi d^3 \rho_i g$$

μ = fluid viscosity

v_t = settling velocity

ρ_f = fluid density

ρ_i = particle density

g = gravitational acceleration

Terminal velocity "v_t" is the velocity at which the microbead is settling at constant speed and is no longer accelerating. Do a force balance and solve for "v_t" in terms of variables.

Cell Interactions and Hindrance:

The settling velocity for each particle of species "i" must be corrected for: 1) average suspension density and 2) viscous effects due to particle collisions:

$$v_i = \frac{d_i^2(\rho_i - \rho_f)}{18\mu} \times g \times \text{density difference correction} \times \text{viscosity correction}$$

$$\downarrow \qquad\qquad \downarrow \qquad\qquad \downarrow$$

$$v_i = S_i \times g \times \frac{\rho_i - \bar{\rho}_{susp}}{\rho_i - \rho_f} \times \varepsilon_i^{4.6}$$

Notes:

- "S_i" is the sedimentation coefficient, which is the ratio of the terminal velocity to the gravitational acceleration and can further be defined as the

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- The viscosity correction factor is $\frac{\mu}{\mu_{i,susp}} = \varepsilon_i^{4.6}$, where "$\mu_{i,susp}$" is the viscosity that

_____

_____

_____

Several models predict microbead settling, but here we outline that of Maude and Whitmore (A generalized theory of sedimentation. *Br. J. Appl. Phys.,* 9, p. 477, 1958). The correction terms account for the effects of varied microbead sizes and population densities on apparent void space, and suspension densities both of which impact interparticle collisions.

v_i = settling velocity of species "i"

S_i = sedimentation coefficient of species "i"

g = gravitational acceleration

ρ_i = particle density of species "i"

ρ_f = density of the fluid

$\bar{\rho}_{susp}$= density of the suspension (fluid + beads); $\bar{\rho}_{susp} = \sum \alpha_i \rho_i + \alpha_f . \rho_f$

where $\alpha_{i \text{ or } f}$ are the volume fractions of particles or fluid, respectively.

ε_i = effective porosity or void space of species "i"

$$v_i = S_i * g \left(\frac{\rho_i - \bar{\rho}_{susp}}{\rho_i - \rho_f} \right) \varepsilon_i^{4.6}$$

The effective porosity that each species experiences "ε_i" represents the impact of interparticle collisions on viscous drag forces:

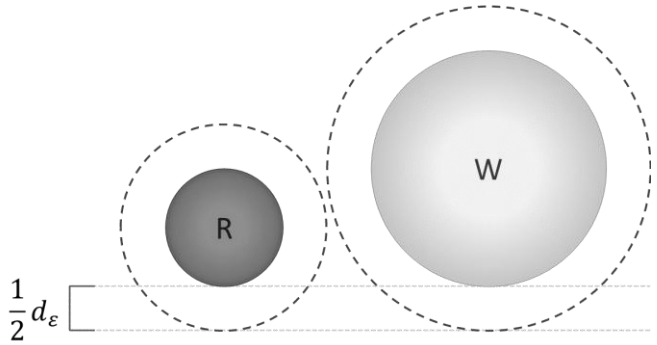
$$\varepsilon_i = 1.0 - \left(1.0 + \frac{d_\varepsilon}{d_i}\right)^{-3} \qquad d_\varepsilon = d_{avg}((1.0 - \varepsilon)^{-\frac{1}{3}} - 1.0)$$

$$\varepsilon = 1.0 - (\text{packed bead } \%) \times (1 - \text{interparticle space})$$

interparticle space = 0.40 for hard spheres

$$d_{avg} = \frac{\sum c_i d_i}{\sum c_i}$$

where d_ε is the average void envelope thickness, ε is the overall void space, and d_{avg} is the average particle diameter in the suspension.



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Why does the larger diameter bead, WBC, settle _____ in the dilute regime (low population density)?

_____

_____

Why does the larger diameter bead, WBC, settle _____ in the dense suspension (high pop. density)?

_____

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