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ABSTRACT

AN INVESTIGATION OF CENTRIFUGAL BLOOD-CELL SEPARATION

Timothy Edward Roche

This dissertation investigated the centrifugal, batch separation of whole blood into subpopulations of red blood cells (erythrocytes) and white blood cells (leukocytes). Separations took place in a custom-built centrifuge (using a seal-less, anti-twisting feed/withdrawal system) containing a 25-ml capacity separation chamber. The blood separation chamber had a dart-shaped geometry in the radial plane and a constant depth in the axial direction. Separation experiments were performed on whole bovine blood at varying hematocrit, centrifuge speed, and batch duration. A small, companion study of whole human blood separation runs also were conducted; they concentrated on batch duration effect and achieved superior separations.

A new graphical technique—generating accumulated cell-fraction separation graphs and measuring separation quality—was devised to display experimental separation runs. Results were presented for both bovine blood and human blood. An interval, observable between the accumulated cell-fraction curves of red blood cells and white blood cells, was measured and used to quantify the maximum extent of separation, allowing for determination of good and bad separations. This measured value was labeled separation quality (SQ). Measurements of SQ for bovine blood separation runs of various duration showed that batch duration had a strong correlation to separation quality. The set of human blood separation runs demonstrated that SQ values may be used as a means to locate optimal operating parameter values. An optimum was bounded for the human blood data set. A one-dimensional volume-diffusion model has been derived for the equations of change of fluid mechanics. The volume-diffusion model extended the original work of Bird, Curtiss, and Hirshfelder in the area of molecular diffusion to application on particulate systems where volume diffusion was the predominant driving force. This model described the binary system of red blood cells (erythrocytes) and plasma. Expressions for volume flux with respect to stationary coordinates, including contributions via ordinary diffusion and pressure diffusion, were derived from the molecular flux expressions for the corresponding diffusion contributions. Due to its high degree of complexity, the model's system of partial differential equations could not be solved using a collocation finite element solver. The model was intractable.

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AN INVESTIGATION OF CENTRIFUGAL BLOOD-CELL SEPARATION

Timothy Edward Roche Dr. Sam S. Sofer, Advisor

A Dissertation Submitted to the Faculty of New Jersey Institute of Technology in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemical Engineering

> Department of Chemical Engineering, Chemistry, and Environmental Science

> > May 2001

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To my family:

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CHAPTER 1 RATIONALE

1.1 Blood Overview

Blood is vital to life. Its function is homeostasis: to maintain the constancy of the internal environment for the body. The essential tasks it provides vary in importance from imperative for life to necessary for health. Blood regulates the body's pH levels (about 7.4), facilitating bodily chemical reactions; blood delivers oxygen and removes carbon dioxide, allowing cellular respiration (without which cells begin to die in 3 minutes); blood controls body temperature, it is our heat transfer fluid keeping the body core not too hot or not too cold; blood provides nutrients to every cell, blood sugar (one of many nutrients) is the primary fuel for the brain, spinal cord, nerves, and the only food source for red blood cells (which have no mitochondria); blood disposes of waste materials (excretion); blood defends the body—resisting or destroying and then removing foreign organisms, dead cells, and other foreign materials, conferring immunity from infectious agents, producing the inflammatory response, sealing and repairing wounds—its defensive mechanisms are achieved by a complex interaction of blood cells and plasma constituents. Truly a remarkable tissue.

Blood is the only tissue in the body which is liquid: a mélange of several types of specialized cells or formed elements (45 vol%) suspended within liquid plasma (55 vol%). This percentage is called the hematocrit. There are three key types of blood cells: red blood cells (RBCs or erythrocytes), white blood cells (WBCs or leukocytes), and platelets (thrombocytes). A collection of tables with details on blood and its constituents

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are included in Appendix E. At the end of this chapter one may review concentrations and sizes of many blood constituents in Table 1-1.

Plasma is the liquid portion of blood. It contains 90% water. By far the largest body constituent, water is essential to the life processes of every cell and it is freely exchanged with body cells and other extracellular fluids via the blood vessels. Plasma contains not just water but proteins, carbohydrates, and fats: to a lesser extent, electrolytes, organic acids, and non-protein nitrogenous compounds: and in very small amounts, vitamins, hormones, and enzymes. By weight, proteins are 7% of plasma. The plasma proteins exert an osmotic pressure which tends to move water from other extracellular fluids to the plasma. Albumins (4.8 g/ml), globulins (2.5 g/ml), and fibrinogen (0.3 g/ml) constitute the chief proteins by concentration. Plasma is a complex solution.

Red blood cells (RBCs) or erythrocytes are the most prevalent cells or formed elements in blood. (RBCs do not have nuclei, so technically they are not cells.) They account for 95% of blood cells by number. A human RBC have the form of a biconcave disc (like a donut with an incomplete hole), a shape that provides a large surface-tovolume ratio. , except when flowing through capillaries where it is squeezed into a bullet shape because many capillaries are smaller than the cell diameter. Red blood cells may flocculate one cell on top of another, not unlike stacked coins, forming a rouleau structure. The principal function of RBCs is gas transport: O_2 delivery and CO_2 removal. The transport of both, O_2 and CO_2 , is facilitated by the presence of hemoglobin^{*} (a protoporphyrin-iron-globin complex), which readily associates with either O_2 and CO_2 . The average life span of a RBC is 120 days.

White blood cells (WBCs) come is a variety of types, each with its separate functions. They account for 0.13% of blood cells by number. Red blood cells out number WBCs approximately 1000 to 1. The WBCs are often classified, based on the presence of granules in their cytoplasm, as either granulocytes (cells with granules) or agranulocytes (cells sans granules). Another name for granulocytes is polymorphonuclear leukocytes (white blood cells with a multi-lobed nucleus). Granulocytes account for about 70% of all WBCs. They are formed in the bone marrow. Three types of granulocytes may be distinguished, by names based on the color of the cell's granules when stained with a compound dye: neutrophils (granules stain pink, vast majority of granulocytes), eosinophils (granules stain red, 2% of granulocytes), and basophils (granules stain blueblack, 1% of granulocytes). Agranulocytes include monocytes and lymphocytes.

Platelets (or thrombocytes) are the smallest cells of the blood $(2-4\mu m)$, diameter). Even though platelets out number WBCs approximately 40 to 1, they occupy a much smaller fraction of the volume fraction. Like RBCs, they lack a nucleus and are consequently incapable of cell division, but differ from RBCs by virtue of a more complex metabolism and internal structure. Hemostasis, the prevention and control of bleeding, is the task of platelets. This function is aided by their ability to adhere to each

[•] The hemoglobin molecules (68,000 mol wt) are extremely tightly packed within the RBC; they constitute about 25% of the available volume, the remainder being taken up by water (70%) and other constituents

other but not to RBCs or WBCs. Within the platelet are tiny granules that contain clotpromoting substances. The largest bone marrow cells, megakaryocytes, produce the smallest blood cells, platelets. By the time they reach circulation platelets will remain for about 10 days before being removed and destroyed.

1.2 Reasons to Separate Blood

Blood is necessary to life. (One need only hold one's breath to be reminded of blood's role in delivering oxygen to and removing carbon dioxide from the various organs, tissues, and diverse cells throughout the body.) And so all reasons for separating blood ultimately may be reduced to the universal desire to continue living. Effective techniques for separating, concentrating, and accumulating one or more components of blood can only improve our ability to understand blood's properties, to ascertain the state of an individual's health, and to treat diseases of the body. (1) Scientific knowledge, (2) diagnostic testing, (3) therapeutic treatment are three basic reasons for separating blood.

1.3 Effective Blood Separation

Previous work by graduate students at the University of Oklahoma under the direction of Dr. Sofer resulted in the construction of a centrifuge incorporating an anti-twister mechanism, enabling the delivery of blood to and from the separation chamber of the centrifuge while avoiding the constraint of passing through rotating seals (potentially causing hemolysis due to mechanical stress on cells). With this existing custom

^{(5%).} One gram of hemoglobin can combine with 1.34 ml of O_2 . Hence, at a hemoglobin concentration of

¹⁵ g/100 mL blood, the O_2 -carrying capacity of blood is about 20 volume-percent (Kline 1976).

centrifuge available, it was natural to desire a better understanding of its separation qualities and, reaching that goal, advance the cause by seeking improvements.

The search for potential improvements focused first on easily adjustable centrifuge operating conditions—varying initial hematocrit, centrifuge speed, batch duration—and the focus would later shift to more substantial (laborious, costly) changes. Specifically, these changes might entail designing and constructing centrifuge chambers of superior geometry and perhaps devising more elaborate separation schemes (implementing more complicated fill/withdrawal patterns and/or adding additional chambers), the result being a staged version of the separation process.

Experimental studies of the existing chamber and its variable operating conditions may progress only so far until further improvement will be stymied, the hindrance is the fixed geometry of the chamber. Newly shaped chamber geometries may either be investigated experimentally, after building and installing a new chamber shape, or be investigated theoretically, after modeling the separation phenomena and solving the simulation for a given chamber shape's separation effectiveness. The experimental route is costly in terms of time and material and labor. So a good model predicting the behavior of blood undergoing centrifugation is a goal for the complete investigation of blood cell separation.

	Concentration	Shape	Size or Mol. Weight
Cellular Elements			
Erythrocytes	5×10^{6} cells/mm ³	discoid	$8\mu m$ (diameter)
Leukocytes	5 to 8×10^3 cells/mm ³		
monocytes		polymorphous	$16-22 \mu m$ (diameter)
granulocytes		polymorphous	$10-12 \mu m$ (diameter)
lymphocytes		spherical	$7-12\mu m$ (diameter)
Platelets	2.5 to 5×10^5 cells/mm ³		$2.5\mu m$ (diameter)
Plasma Molecular d	constituents		
Albumin	3.5–5.3 g/100mL	prism	69×10^3 g/mol
Globulin	2.1-3.3 g/100mL	ellipsoid	$(41-1000) \times 10^3$ g/mol
Lipoprotein		spherical	$(200 - 13000) \times 10^3$ g/mol
Fibrinogen	0.2–0.4 g/100mL	dumbbell	400×10^3 g/mol
Glucose	70–120 mg/100mL		180 g/mol
Ionic Content of Pla	isma		
Na^+	145 mEq/L		
K^+	4.2 mEq/L		
Ca ⁺²	4.8 mEq/L		
Cl⁻	103 mEq/L		
HCO ₃	29 mEq/L		
HPO_4^{-2}	2 mEq/L		
Other cations	6 mEq/L		
Other anions	21 mEq/L		

 Table 1-1 Composition of blood (Kline 1976, 142).

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CHAPTER 2

INTRODUCTION

2.1 Settling and Sedimentation in Particle-Fluid Separation

When a particle is at a sufficient distance from the walls of the container and from other particles so that its fall is not affected by them, the process is called *free settling*. Interference is less than 1% if the ratio of the particle diameter to the container diameter is less than 1:200 or if the particle concentration is less than 0.2 vol% in the solution. When the particles are crowded, they settle at a slower rate and the process is called *hindered settling*. The separation of a dilute slurry or suspension by gravity settling into a clear fluid and a slurry of higher solids content is called *sedimentation* (Geankoplis 1993).

2.1.1 Leukapheresis

Hemapheresis, the selective collection of any blood component, has become practical with the use of automated equipment—both for obtaining specific components for transfusion, and for removing pathogenic components in clinical disease (Silvergleid 1983). A large number of diseases, primarily with an immunological basis, have been treated in this way, but not without adverse reactions (Kennedy and Domen. 1983; Westphal 1984). Recent reviews of plasmapheresis, cytapheresis, and blood cell separators are enlightening (Blumberg and Katz 1981; Tan and others 1981).

Continuous flow centrifugation technology is at an advanced state — the automated, rapid, efficient, and relatively safe procedures are well tolerated by patients.

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While the clinical benefit of leukapheresis in chronic myelocytic leukemia is not conclusive, it can reduce by one-half the WBC count within two or three hours — a task which may take chemotherapy several days (Mallard 1982).

One function of a centrifugal detoxification scheme is to return healthy granulocytes to the patient. For patients suffering from hematological malignancies (Hersh and others 1965; Cheng and others 1976; Russell and Powles 1976) and solid tumors (Ingaki, Rodriguez, and Bodey 1976) infection remains as the leading cause of death. In most cases insufficient numbers of functional granulocytes appear to be the major deterrent in warding off infection (Bodey and others 1975). Improvements in leukapheresis within recent years have aided in establishing granulocyte transfusion therapy as an effective therapeutic modality in controlling infection (Steigbigel and others 1978; Winton and Vogler 1978; Higby and Henderson 1975; Graw and others 1972; Higby and others 1977; Alavi and others 1977).

2.1.2 Sedimentation Agents

In order to increase collection efficiency, erythrocyte sedimenting agents such as dextran (Winton and Vogler. 1978; Aisner, Schiffer, and Wiernik. 1978; Lowenthal and Park. 1975) or hydroxyethyl starch (HES) (Russell and Powles 1976; Winton and Vogler 1978; Aisner, Schiffer, and Wiernik 1978; McCredie and others 1974; Mischler and others 1974) are sometimes used.

These sedimenting agents cause rouleau formation to increase, thereby preferentially increasing the red blood cell sedimentation rate (Mittelman and others 1985), and increasing granulocyte yields to between $1.3 \times 10^{10} - 5.2 \times 10^{10}$ cells (Herzig,

Root, and Graw 1972; Huestis, and others 1975; Sussman and Colli 1975; McCredie, and others 1975; Benbunan, and others 1975).

Sedimentation agents are an added complication in a detoxification process. Strauss (1981) has shown overt bleeding and hemorrhaging following large doses of HES. Consequently no sedimentation agents or other similar chemicals are used with the blood processing technique.

2.1.3 Filtration Techniques

Filtration leukapheresis has also been used to increase cell yields. But donors undergoing filtration leukapheresis often exhibit profound neutropenia sometimes accompanied by chills, hypotension, and mild respiratory symptoms (Hammerschmidt and others 1978). One might suspect that the neutropenia may be explained on the basis of filter trapping of granulocytes, however the decline is much too sharp to be attributed to the filter alone (Hammerschmidt and others 1978). Herzig and coworkers (1975) have attributed the effect to complement activation resulting when plasma contacts the polymer surfaces inside the filter. Once reinfused into the donor the activated complement causes granulocytes to aggregate and to be sequestered in the lungs causing demonstrable lung dysfunction. Filtration techniques have therefore also been ruled out for the blood processing scheme in this investigation.

2.1.4 Shear Damage and the Anti-twister Mechanism

Complement activation problems have long been reported for hemodialysis (Kaplow and Goffinet 1968). Jacob (1983) reports activation during extracorporeal circulation leading

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to leucoembolization, granulocyte aggregation, and physical problems ranging from modest pulmonary dysfunction to severe pulmonary damage.

In extracorporeal shunts, blood is generally forced through rotating seals, exposing the components to high shear stresses. RBC's and WBC's are damaged, and platelets (Brown, Lemuth, and others 1975; Brown, Leverett, and others 1975) are particularly susceptible to damage caused by rotating seals (Suaudeau, and others 1978; Ito, Suaudeau, and Bowman 1977).

Even in low-flow, short-term operations, sufficiently high levels of heparin and citrate dextrose anticoagulants must be maintained in the centrifuge bowl to prevent platelet aggregation (Suaudeau, and others 1978; Ito, Suaudeau, and Bowman 1977; Graw and others 1971; Hester, McCredit, and Freireich 1975; Ruder and Wilson 1975). In higher-flow, longer-term processes, increased amounts of anticoagulants would be necessary, thereby increasing the potential for bleeding problems or citrate reactions (Suaudeau, and others 1978). In fact, occasional citrate reactions (Huestis, and others 1975; Szymanski, Patti, and Kilman 1973; Oon and Hobbs, *Clinical applications*, 1975; Oon and Hobbs, *Medical problems*, 1975) are observed even in low-flow, short-term processes.

Besides the platelet problem, numerous other complications have been encountered including frequent leakages and inter-communication between blood fractions through the rotating seal (Suaudeau and others 1978). These complications were found to be a consequence of uneven lubrication, swelling and displacement of seal O-rings, or even sudden changes in animal venous pressure due to postural changes, sneezing or coughing (Suaudeau, and others 1978). A more recent development in continuous centrifuge design developed by Ito and Kolobow (Ito, Suaudeau, and Bowman 1977) of NIH, circumvents the need for rotating seals, allowing attachment of continuous tubes directly to the rotating centrifuge head. Tubes are kept from twisting by an anti-twister mechanism (Ito, Suaudeau, and Bowman 1977; Ito 1976). An additional feature: the centrifuge head contains a blood chamber of spiral design which allows for adequate separation and mixing of adjacent sedimentation layers, thereby increasing separation efficiency. Kolobow, Ito, and Suaudeau of NIH have performed preliminary comparative studies with the seal-less centrifuge and a centrifuge with rotating seals (Aminco Celltrifuge) (Suaudeau, and others 1978). The seal-less design showed remarkable improvements over the Aminco Celltrifuge, which maintained continuous operation for 24 hours at almost twice the rpm and twice the flow rate with little or no platelet damage or adverse reactions on experimental animals (Suaudeau, and others 1978; Ito, Suaudeau, and Bowman 1977).

Preliminary centrifugation experiments by Kolobow (1978), although successful in separating plasma and platelets from erythrocytes, were not successful in producing granulocyte harvests at high yields, and no rigorous attempts were made to study optimum operating conditions for granulocyte harvesting.

It was with the close cooperation of Kolobow and Ito that Sofer and Van Wie constructed several blood processors incorporating the anti-twister mechanisms and the spiral chamber. The more recent versions (V-3, now at NJIT) are machined to closer tolerances, and equipped with dynamic balancing systems.

The anti-twister device allows greater flow rates and operation at higher g forces allowing for increased efficiency, resolution, and throughput. This will help offset the fact that sedimentation and density gradient agents are not used in our process.

2.2 Early Work

Our research group first considered centrifugation to separate cells as an adjunct to extracorporeal detoxification using liver enzyme systems to purify plasma. Next, we started work on a device to produce lymphokine subsets in a sedimentation chamber. At an upward flow of fluid equal to the sedimentation velocity, we obtain a gravity-immobilized white cell bioreactor. Early work in this laboratory, supported by NIH, the University of Oklahoma, the State of New Jersey and other sources, led to the development of a centrifugal blood processing technique of high efficiency and resolution. We tested staging, recycle, and many heads and chamber shapes.

The V-3 blood processor was constructed after Bernie Van Wie (then a student of Dr. Sofer at the University of Oklahoma) spent some time as a guest worker with Dr. Ito and Dr. Kolobow of the Laboratory for Technical Development of NIH in Bethesda, learning to build and operate this type of machine. We developed two-component (WBC-RBC) material balance calculation techniques for multistage blood processing. Our theoretical design calculations were supported by encouraging experimental results (Van Wie and Sofer. 1986).

Specifically, our group discovered that operating in regions of low hematocrit, introducing a plasma recycle stream, and increasing the number of stages greatly improve blood component separations. Our efforts were centered on separating the white cell components from the red blood cells, hence we developed the design equations for a twocomponent model.

2.3 Sedimentation Theory for Dilute Systems

In the mid-19th century, Stokes studied the motion of immersed bodies in viscous fluids in a gravitational field (Davidson 1976; Delva Separator Company 1978) and developed an equation describing the particle terminal settling velocity. Our group started the theoretical approach by extending this equation to account for blood component sedimentation velocities (Van Wie and Sofer 1984).

Under a centrifugal force field, the effect of earth's gravity usually becomes negligible compared to the centrifugal acceleration for the force of gravity; Stokes' equation becomes:

$$v_i = \frac{2}{9} \frac{r^2 (\rho_i - \rho_f) \theta^2 r_c}{\mu}$$

where:

 v_i is the terminal settling velocity for component i,

r is the particle radius,

 ρ_i is the particle density,

 ρ_f is the fluid density,

 μ is the fluid viscosity,

 θ is the angular velocity, and

 r_c is the radial distance from the axis of rotation.

Several limiting assumptions are inherent in this equation and are detailed elsewhere (Davidson 1976; Maupin 1969).

Sedimentation theory can be compared to experimental results in actual centrifugation processes at least for dilute particle suspensions. However, in blood separations the application of Stokes' sedimentation relationship appears to have some slight inaccuracies even in dilute suspensions (Zwicker 1972). Some of the inaccuracies can be attributed to the fact that cell size and density not only vary slightly from individual to individual, but depend upon age, cellular environment and measurement technique (Davidson 1976; Van Wie and Sofer 1984). Other inaccuracies may appear with changes in viscosity, which may affect sedimentation rate. Viscosity changes with temperature; for non-Newtonian fluids; viscosity also changes with flow rate. Even with all of the potential for inaccuracies, Stokes' law still provides an understanding of sedimentation theory and can be used to determine guidelines for centrifuge design.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Equipment and Materials

The V-3 centrifuge includes a continuous, anti-twisting, feed/withdrawal line. Built on to the head of the centrifuge is a separation chamber shaped like a kite or dart (within the plane of the centrifuge head) with a constant depth in the axial direction. An engineering drawing of the centrifuge head containing the separation chamber is provided in Appendix F.

A Cole-Parmer Digi-Staltic digital flow/dispensing drive (7527-34) with a Masterflex pump head was used to fill the chamber and withdraw samples.

A Sysmex (CC-180) Micro-Cell Counter, a semi-automated hematology analyzer for *in vitro* diagnostic use in clinical laboratories was used for blood analysis. It provided the following eight analysis parameters.

- 1. White Blood Cell (WBC) or Leukocyte Count
- 2. Red Blood Cell (RBC) or Erythrocyte Count
- 3. Hemoglobin (Hgb) Concentration
- 4. Hematocrit (Hct), true relative percentage volume of erythrocytes
- 5. Mean Corpuscular (Erythrocyte) Volume (MCV)
- 6. Mean Corpuscular (Erythrocyte) Hemoglobin (MCH)
- 7. Mean Corpuscular (Erythrocyte) Hemoglobin Concentration (MCHC)
- 8. Platelet (PLT) Count

Sysmex Quicklyser (QL-20) was used in the preparation of WBC samples for the

blood counter. It is a lysing and hemoglobin reagent.

Sysmex Manoresh (MR-50) detergent was used by the blood counter in its automatic cleaning cycle and for cleaning the centrifuge chamber after separation runs.

Sysmex disposable sample beakers (DB-1) were used during dilution preparation steps for analysis of blood samples.

A Digistrobe stroboscope/tachometer is used to determine and set centrifuge speed.

3.2 Directions for Operating V-3 Batch Blood Centrifuge

Here is a step-by-step list of instructions for operating the V-3 centrifuge.

- 1. Turn on the power: power switch is located on rheostat.
- 2. Clean separation chamber. If the chamber is empty, fill the chamber with detergent solution. Empty the detergent from the chamber and rinse with water; repeat the rinse at least two times (additional rinses may be necessary if debris is visible inside the chamber).
- *Note:* in order to empty the contents from the separation chamber, the centrifuge must be spinning with sufficient speed for the contents to accumulate at the bottom of the chamber where the feed/withdrawal line connects to the centrifuge head. This allows the suction head of the pump to work.

1. Charge the chamber with a measured quantity of blood (maximum capacity, 25 ml) of a known hematocrit. The initial hematocrit may be adjusted to lower values by diluting with an isotonic solution. We use Acid Citrate Dextrose (ACD^{*}) solution for bovine blood; heparin-treated vacuum tubes are used for human blood. Using either ACD solution or heparin arrests the natural coagulating properties of whole blood.

3. If the centrifuge is not spinning at the desired angular velocity, adjust centrifuge spin speed and verify using the strobe light, then start measuring the duration of the batch separation with a stop watch.

^{*} ACD anticoagulant consists of 3.95 g citric acid, 10.9 g sodium citrate, and 12.1 g dextrose (all ingredients anhydrous) diluted in 407 ml distilled water. This quantity is sufficient for treating one gallon of bovine blood.

4. The separation run may be ended when a desired batch duration has been reached or when a separation front between plasma and RBC (close observation will identify WBC in the buffy coat) has become visible using strobe light or if the separation front has traveled a desired distance down the chamber.

Note: orienting in the spinning chamber: in order to facilitate discussion of blood cell separations and movement of cells in the chamber, we stipulate that the directions up and down refer to movement, relative to the chamber boundaries, along radial lines, such that:

Up, describes movement on a radial line toward the axis of rotation;

Down, describes movement on a radial line away from the axis of rotation.

- 5. Collect samples. This is achieved by withdrawing the contents of the chamber, effectively from bottom to top, because the withdrawal line is located at the bottom and therefore the bottom fluid leaves first, the top fluid leaves last—using the pump and emptying the drawn off blood into waiting test tubes. Divide the withdrawn contents of the chamber into six or seven separation fractions: accumulate the first 3 ml of blood withdrawn into the first sample test tube, the next 3 ml into the second test tube, and so on, until the chamber is completely empty.
- 6. Count the cells in each separation fraction.
- 7. Plot the results.

3.3 Directions for using a Sysmex[™] (AD-241) Auto Dilutor

3.3.1 WBC Dilution Method

- 1. Turn auto-dilutor power switch to 'ON' position.
- If the instrument is new and operating for the first time, or operating after non-use for several weeks, repeat aspiration and delivery functions 10 times continuously in order to replace the diluent remaining in the hydraulic lines.
- 3. Set the select lever to WBC.
- 4. If a drop of diluent adheres to the pipette tip (sample and bypass pipettes), remove with a piece of gauze or lintless tissue.

- 5. Immerse the tip of the sample pipette into the mixed blood sample.
- 6. Press the Start Button immediately to initiate sample aspiration.
- 7. Remove the blood from the pipette tip immediately after aspiration is completed.
- 8. Remove the blood sample coating the outside of the sample pipette by wiping with damp gauze or damp lintless tissue. Surrounding the upper part of the sample pipette with the gauze or tissue, wipe immediately with a downward motion. *Caution*: If the gauze touches the sample pipette tip, it will cause a dilution error since a portion of the sample inside the pipette may be removed by being soaked into the gauze.
- 9. Place the pipette tips of the sample and bypass pipettes into an unused sample beaker and push the Start Button. The aspirated blood sample and diluent are discharged simultaneously through the pipettes into the beaker.
- 10. Because the output pressure of the pipette is considerable, it is recommended to tilt the beaker so the liquid jet from the pipette tip runs along the beaker's inside wall. This will help maintain cellular integrity.

Caution: Do not immerse the tip of the dilutor pipette into the diluted sample in the tilted beaker. If the blood sample adheres to the inside wall of the sample beaker, resuspend sample with a swirling motion.

11. The diluted sample need not be mixed again if the counting is done immediately because the blood sample is mixed well during the delivery process. Counting shall be started after removing the air bubbles.

3.3.2 RBC Dilution Method

- 1. Set the select lever to RBC.
- 2. Follow the operating procedure described in WBC Dilution Method.

Note: the Dilution Ratio Select Lever can only be changed to the other position when "ASP" is observed through the Monitor Window. The Dilution Ratio Select Lever must never be changed or shifted during the operation of the dilutor or after the completion of the aspiration process. ("DIL" will be observed through the Monitor Window when aspiration is completed.)

3.3.3 Diluent

- 1. Temperature of the diluent should be maintained at approximately 25°C for stable counting of white cells.
- 2. Cycle the dilutor to remove any air in the hydraulic lines when the diluent container is replaced by a new one, or whenever air is observed in the pipette.
- 3. After delivery, a small volume of diluent may remain on the tips of the pipettes, but this will not affect counting result.

CHAPTER 4

RESULTS

4.1 Data Preparation

In the course of a blood separation run, the operator fills the centrifuge chamber with approximately 20–25 ml of blood, spins the blood for a measured duration, and then withdraws the separated blood into accumulated fractions of approximately 3 ml each. (The withdrawal process has the effect of emptying the chamber from the bottom to the top with very little mixing of the remaining blood in the chamber). The blood cells in each collected fraction are counted. A typical output from the counter would resemble Table 4-1, the results of blood cell counts for run B19881212a. All runs are described in detail in Table 4-4.

Sample	Volume	WBC	PBC	TICT		
			KDC	HUI	PLI	Time
	(μL)	concentration	concentration	(%)	concentration	(s)
		$(10^3/\mu L)$	$(10^{6}/\mu L)$		$(10^3/\mu L)$	
1	2500	3.60	13.20	66.0	652	120
2	2500	11.40	14.56	73.0	670	65
3	3500	4.50	9.60	47.0	771	43
4	3000	2.70	8.68	42.9	765	36
5	3000	2.40	7.61	37.0	866	37
6	2500	10.00	5.05	25.0	961	40
7	3000	0.94	0.30	1.4	100	40
initial	20000	4.40	7.80	38.8	555	1560

 Table 4-1 Blood data* and cell counter analysis output.
Table 4-1 includes seven rows of data for the collected fractional samples of the separated blood and one row for the original homogenous blood. The column labeled Volume reports the sample volume (or initial chamber volume) in microliters. WBC concentration is reported in thousands of cells per microliter, as are platelet concentration; the RBC concentration is reported in millions of cells per microliter. Hematocrit (Hct) is the volume fraction of RBCs reported as a percentage.

$$Hct = \frac{RBC \text{ volume}}{\text{whole blood volume}} \cdot 100$$

Time refers to the sampling duration in seconds needed to withdrawal the sample (or the duration of the batch prior to withdrawal in the case of the last row of the table, labeled initial).

The actual cell concentrations are not graphed, rather, values derived from them are graphed: accumulated cell fractions. The three necessary values are volume fraction, WBC fraction, and RBC fraction. The volume fraction is calculated by dividing each individual sample volume (V_i) by the sum of all the sample volumes, e.g., volume fraction of sample 1 (Vf_1) is the result of this equation: $Vf_1 = V_1 / \sum_{i=1}^{7} V_i$. Using the volume data from Table 4-1, the value for Vf_1 may be calculated, as follows.

* Example data from separation run B19881212a.

$$V_{1} = 2500$$

$$\sum_{i=1}^{7} V_{i} = (2500 + 2500 + 3500 + 3000 + 3000 + 2500 + 3000) = 20000$$

$$Vf_{1} = \frac{V_{1}}{\sum_{i=1}^{7} V_{i}} = \frac{2500}{20000} = 0.1250$$

Fractions—volume, number, or otherwise—have the advantage of being dimensionless quantities. The calculated volume fraction is also a normalized value—the denominator is the sum of the sample volumes rather than the original total chamber volume (the two volumes are not necessarily the same value). Normalizing ensures that the fractions sum to one.

Similarly, the WBC- and RBC-number fractions are calculated by analogous formulas, replacing sample volume with the product of sample volume and WBC concentration to calculate WBC-number fraction; likewise, RBC concentration to calculate RBC-number fraction. Here is an example calculation to find the WBC-fraction for sample 1 ($WBCf_1$), using volume and WBC concentration data from Table 4-1.

$$V_{1} \cdot WBC_{1} = 2500 \cdot 3.60 = 9000$$

$$\sum_{i=1}^{7} V_{i} \cdot WBC_{i} = \begin{pmatrix} 2500 \cdot 3.60 + 2500 \cdot 11.40 + 3500 \cdot 4.50 + 3000 \cdot 2.70 \\ +3000 \cdot 2.40 + 2500 \cdot 10.00 + 3000 \cdot 0.94 \end{pmatrix}$$

$$= (9000 + 28500 + 15750 + 8100 + 7200 + 25000 + 2820) = 96370$$

$$WBCf_{1} = \frac{V_{1} \cdot WBC_{1}}{\sum_{i=1}^{7} V_{i} \cdot WBC_{i}} = \frac{9000}{96370} = 0.0934$$

Here is the equivalent example calculation to find the RBC-fraction for sample 1 $(RBCf_1)$, again, using volume and RBC concentration data from Table 4-1.

$$V_{1} \cdot RBC_{1} = 2500 \cdot 13.20 = 33000$$

$$\sum_{i=1}^{7} V_{i} \cdot RBC_{i} = \begin{pmatrix} 2500 \cdot 13.20 + 2500 \cdot 14.56 + 3500 \cdot 9.60 + 3000 \cdot 8.68 \\ +3000 \cdot 7.61 + 2500 \cdot 5.05 + 3000 \cdot 0.30 \end{pmatrix}$$

$$= (33000 + 36400 + 33600 + 26040 + 22830 + 12625 + 900) = 165395$$

$$RBCf_{1} = \frac{V_{1} \cdot RBC_{1}}{\sum_{i=1}^{7} V_{i} \cdot RBC_{i}} = \frac{33000}{165395} = 0.1995$$

Table 4-2 Volume and number fra	actions, normalize	ż۵.
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Sample	Volume	WBC fraction	RBC fraction	Hematocrit	Platelet
	fraction	(WBCf)	(RBCf)	fraction	fraction
	(Vf)			(HCTf)	(PLTf)
1	0.1250	0.0934	0.1995	0.2016	0.1199
2	0.1250	0.2957	0.2201	0.2230	0.1232
3	0.1750	0.1634	0.2032	0.2010	0.1984
4	0.1500	0.0841	0.1574	0.1573	0.1688
5	0.1500	0.0747	0.1380	0.1356	0.1910
6	0.1250	0.2594	0.0763	0.0764	0.1767
7	0.1500	0.0293	0.0054	0.0050	0.0221
Material balance ratio	1.0000	0.9131	0.9432	0.9483	0.8162

The material balance ratio is the result of dividing the original quantity by the summation of the sample quantities—in/out—yielding a check on the material balance of the batch separation process. Ratios greater than one indicate more was measured going in than was measured coming out; ratios less than one signify the opposite; a ratio equal to one is material balanced. For instance, the material balance ratio for the WBC-number fraction column corresponds to the total WBCs initially charged into the

[†] Continuing the example for separation run B19881212a, most of the required data is taken from Table 4-1.

separation chamber divided by the summation of the total WBCs in each sample. Here is the calculation of the WBC material balance ratio. (In the calculation V_0 and WBC_0 refer to the initial volume and initial WBC concentration, respectively.)

$$V_{0} \cdot WBC_{0} = 20000 \cdot 4.40 = 88000$$

$$\sum_{i=1}^{7} V_{i} \cdot WBC_{i} = \begin{pmatrix} 2500 \cdot 3.60 + 2500 \cdot 11.40 + 3500 \cdot 4.50 + 3000 \cdot 2.70 \\ +3000 \cdot 2.40 + 2500 \cdot 10.00 + 3000 \cdot 0.94 \end{pmatrix}$$

$$= (9000 + 28500 + 15750 + 8100 + 7200 + 25000 + 2820) = 96370$$

$$\frac{\text{in}}{\text{out}} = \frac{\text{initial WBCs}}{\sum \text{sample WBCs}} = \frac{V_{0} \cdot WBC_{0}}{\sum_{i=1}^{7} V_{i} \cdot WBC_{i}} = \frac{88000}{96370} = 0.9131$$

The resulting WBC material balance ratio, 0.9131, indicates that the initial WBCs are nearly 10 percent less than the sum of the sample WBCs—this is only a borderline acceptable WBC material balance. It preferred that material balance between feed and withdrawal agree within 10 percent. The RBC material balance ratio, 0.9430, is much better. Notice, in Table 4-2 that the material balance ratio for platelets is 0.8162, an unacceptable value, but we are not interested in platelet counts for this investigation. (The platelet counts for bovine blood are notoriously unreliable, this is likely a result of using a blood counter tuned for human blood rather than cow blood).

To construct the accumulated cell-fraction graphs, accumulated fractions are needed for volume fraction, WBC fraction, and RBC fraction. An accumulated numberfraction or accumulated volume-fraction for a given sample i, follows this simple formula. Let ' Σ Fraction' stand for a generic, accumulated fraction (either volume or cell number).

$$\Sigma$$
Fraction_i = \sum_{1}^{i} Fraction_i

For example, the accumulated volume-fraction (ΣVf) for sample 1 is just the volume fraction of sample 1, $\Sigma Vf_1 = Vf_1$. But, the accumulated volume-fraction for sample 4 is the sum of volume fractions of samples 1 through 4, $\Sigma Vf_4 = Vf_1 + Vf_2 + Vf_3 + Vf_4$. Imagine pouring the first four fractionated samples, 1 through 4, into a container; that container would hold the accumulated cells, and the sum of those cells divided by the original total cells would yield the accumulated cell-fraction. Of necessity, the accumulated fraction for the last sample will always sum to one, because the sample fractions are normalized.

			•	
Sample	Accumulated Volume-Fraction $(\Sigma V f)$	Accumulated WBC-Fraction $(\Sigma WBCf)$	Accumulated RBC-Fraction $(\Sigma RBCf)$	$\Delta = \Sigma RBCf - \Sigma WBCf$
1	0.1250	0.0934	0.1995	0.1061
2	0.2500	0.3891	0.4196	0.0305
3	0.4250	0.5526	0.6228	0.0702
4	0.5750	0.6366	0.7802	0.1436
5	0.7250	0.7113	0.9182	0.2069
6	0.8500	0.9707	0.9946	0.0238
7	1.0000	1.0000	1.0000	0.0000
	Maximu	m Δ = Separatio	on Quality (SQ)	0.2069

 Table 4-3 Accumulated volume and cell number fractions‡.

Table 4-3 lists values for accumulated volume-fraction and accumulated cellfractions (RBC, WBC) for each fractionated sample. Also, listed is a column of cell fraction differences entitled $\Delta = \Sigma RBCf - \Sigma WBCf$; the values in this column are the difference between the accumulated RBC-fraction ($\Sigma RBCf$) and the accumulated WBC- fraction ($\Sigma WBCf$). This cell fraction difference (Δ) quantifies the vertical distance between the plotted data points (usually RBC point above and WBC point below) for an individual sample data set, in other words.

$$\Delta_i = \Sigma RBCf_i - \Sigma WBCf_i$$

The best cell fraction difference (Δ_{max}) for a run is designated the separation quality (SQ) for the run. This reflects the practical situation, for the sample at which the accumulated cell-fraction difference is greatest is where one would define the separation cut for the run. Everything accumulated up to the cut in one bucket, everything accumulated after the cut in another bucket.

With the accumulated volume-/cell-fractions compiled the only remaining task is graphing the results. The graph for the example separation run, B19881212a, used in Table 4-1, Table 4-2, and Table 4-3 is found in Figure 4-7, below.

[‡] Continuing the example for separation run B19881212a, most of the required data is taken from Table 4-1 and Table 4-2.

4.2 Discussion



Figure 4-1 Hypothetical, ideal, blood-cell separation. This ideal separation would yield a separation quality, SQ=1.0.

4.2.1 Ideal Separation

Consider a blood separation, RBCs from WBCs, where the population of RBCs is completely segregated from WBCs, and let the population of RBCs settle into a bed of cells at the bottom of the chamber with a packing factor of 1.0—the RBCs have squeezed everything else out of the packed bed—no plasma, no WBCs, no platelets.

This accumulated cell-fraction graph, Figure 4-1, represents a theoretical, maximum separation for a blood sample with starting hematocrit of 45 percent. The RBC line assumes an unreachable RBC packing factor of 1.0 and complete separation between

RBCs and WBCs, also impossible. Because the initial volume fraction (hematocrit) is 0.45, and because the RBCs are packed to the point of squeezing everything else out (cells, plasma), then the RBC curve will intersect the RBC-fraction=1.0 level at a volume fraction equal to the initial RBC volume fraction. The X=Y line serves as a reminder of the opposite extreme—zero separation—if a homogeneous sample were withdrawn, its RBC and WBC curves would lie superimposed upon the X=Y line.

4.2.2 Bovine Blood Summary

The collected bovine-blood separation-run data are summarized in the tables found in Appendix C. For convenience, these data are sorted by run date, initial hematocrit, centrifuge spin speed, batch duration, and separation quality.

4.2.3 Bovine Blood Separation Runs

Initial hematocrit, centrifuge spin speed, batch duration: these are the variables that are investigated in this study. Other parameters that can and do affect the separation of cells are held constant; these include chamber geometry, chamber material of construction, temperature.



Figure 4-2 Bovine blood separation run B19890421a.

Separation run parameters:blood collected 1 day(s) prior to run;initial hematocrit, HCT=37.00;centrifuge spin speed, RPM=910 min⁻¹;batch duration, θ =4740 s;separation quality, SQ=0.4833.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation. This accumulated cell-fraction graph represents the best bovine-blood separationachieved in this investigation.

Batch Duration

In the bovine separation runs, B19890421a, B19890420a, and B19890424a, (graphs found in Figure 4-2, Figure 4-3, and Figure 4-4, respectively) we see three separations, each with similar initial hematocrit, and similar centrifuge spin speed, but with differing batch duration. For these three runs the separations improve with increased batch duration. This is evident in the change of SQ values for the three runs listed in Table 4-4, right-most column.

Run	Graph	Initial hematocrit	Centrifuge spin speed (min ⁻¹)	Batch duration (s)	Separation quality
B19890421a	Figure 4-2	37	910	4740	0.48
B19890420a	Figure 4-3	37	1000	2820	0.39
B19890424a	Figure 4-4	37	1019	2160	0.29

Table 4-4 Varying batch duration separation runs.

General observations for separations

Here are some observations that were found during the performance of the separation runs or while preparing the separation graphs.

Scanning from left to right on an accumulated cell-fraction graph corresponds to moving from the bottom of the separation chamber to the top, because the chamber contents are removed from bottom to top. RBCs dominate the movements of all particle types due to sheer numbers. RBCs out number WBCs approximately 1000 to 1. This is a point to keep in mind while viewing the accumulated cell-fraction graphs. The graphs represent RBCs and WBCs equally as fractions (These values are relative to the total number of cells for a given sub-population, not relative to the total particle population: RBCs compare to total RBCs, WBCs compare to total WBCs.)

Accumulated cell-fraction graphs can usually be divided into two predominant parts: the packed RBC portion and plasma plateau portion. The packed RBC portion starts at zero RBCs removed (at the left most side of the graph, $\Sigma RBCf = 0$) and continues until all the RBCs have been withdrawn (someplace within the interior of the graph, $\Sigma RBCf = 1$). The point at which the packed RBC portion ends is also the beginning of the plasma plateau. The plateau starts within the interior, where the accumulated RBC-fraction first reaches its limit, $\Sigma RBCf = 1$; the plasma plateau extends to the right, out to the limit of the graph. RBCs settle during the dynamic batch and collect in a bed of cells at the bottom of the chamber. As the bed of mostly red blood cells first forms it will have graduated density distribution: densely packed at the bottom of the bed and significantly less densely packed toward the top of the bed. With extended batch duration the RBC bed becomes more uniformly packed, eventually reaching some maximum packing factor and a corresponding maximum volume-fraction of RBCs (hematocrit).





Separation run parameters: initial hematocrit, HCT=37.00; batch duration, θ =2820 s; The X=Y line (dashed) and packed blood collected 0 day(s) prior to run; centrifuge spin speed, RPM=1000 min⁻¹; separation quality, SQ=0.3908.

The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating the separation: the X=Y line corresponds to zero separation; the packed-RBC line represents ideal RBC separation.

Prior to achieving the separation limit for RBCs, while the RBC bed is still forming, the plotted RBC data points will curve reflecting the non-homogeneous bed density at the bottom of the chamber. As the separation reaches its limit and the RBC bed reaches its maximum packed hematocrit, the plotted RBC data points in the accumulated cell-fraction graph straighten out and rotates toward the packed-RBC line. The required duration for the RBC bed to pack is a function of the initial hematocrit (how many cells have to settle?) and centrifuge spin speed (how powerful is the predominant driving force of the pressure gradient?).



Figure 4-4 Bovine blood separation run B19890424a.

Separation run parameters:blood collected 4 day(s) prior to run;initial hematocrit, HCT=38.8;centrifuge spin speed, RPM=1019 min⁻¹;batch duration, θ =2160 s;separation quality, SQ=0.2920.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.

WBC separation curve will generally lag the RBC separation curve. In the best separations one sample point will contain a majority of the WBCs. This large slug of cells is a consequence of the formation of a buffy coat (WBC rich layer) in the chamber between the RBC bed at the bottom of the chamber and the plasma supernatant at the top of the chamber.

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Figure 4-5 Bovine blood separation run B19890427a.

Separation run parameters:blood collected 1 day(s) prior to run;initial hematocrit, HCT=6.43;centrifuge spin speed, RPM=900 min⁻¹;batch duration, θ =2100 s;separation quality, SQ=0.1869.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.

Low Initial Hematocrit

The plasma plateau portion of the accumulated cell-fraction graph is identifiable by the

change in direction of the cell-fraction curves, both of which will become horizontal.



Figure 4-6 Bovine blood separation run B19890614a.

Separation run parameters:blood collected 5 day(s) prior to run;initial hematocrit, HCT=8.02;centrifuge spin speed, RPM=860 min⁻¹;batch duration, θ =1500 s;separation quality, SQ=0.4253.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.

At low initial hematocrit the RBC dominant portion of the graph is shifted to the left and compressed. With a smaller number of cells, there is a smaller RBC bed. The plateau of the plasma dominant portion of the graph is also shifted to the left and the plateau extends across the top of the accumulated cell-fraction graph.

Separations, good and bad, still happen at low initial hematocrit, as illustrated in the two low-initial-hematocrit graphs: good separation run B19890427a in Figure 4-5, and bad separation run B19881212a. in Figure 4-6.



Figure 4-7 Bovine blood separation run B19881212a.

Separation run parameters:blood collected 11 day(s) prior to run;initial hematocrit, HCT=38.8;centrifuge spin speed, RPM=1161 min⁻¹;batch duration, θ =1560 s;separation quality, SQ=0.2069.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating the

separation: the X=Y line corresponds to zero separation; the packed-RBC line represents ideal RBC separation.

Run	Graph	Initial hematocrit	Centrifuge spin speed (min ⁻¹)	Batch duration (s)	Separation quality
B19890427a	Figure 4-5	8.02	860	1500	.4253
B19881212a	Figure 4-6	6.43	900	2100	.1869

Table 4-5 Low initial hematocrit separation runs

Due to the low initial hematocrit these two graphs both appear to be dramatically different from the higher initial hematocrit runs presented in this chapter. But, on closer examination they have all the characteristics of the others, only shifted to the left and compressed because of the vastly smaller proportion of cells relative to the total volume of whole blood.

At low hematocrit values (< 15), characterizing separation runs becomes problematic; not because separations are not occurring, but because the small volume fraction of RBCs, when accumulated and packed by the centrifuge, occupies less than the volume of one sample (around 3 ml). Whether or not a significant separation (large SQ) occurs in the batch chamber, when the first sample is collected from the bottom of the chamber, containing the total volume of RBCs and likely most of the WBCs, all resolution is lost through mixing of the blood components. The subsequent analysis on this sample blurs the actual separation achieved in the chamber by re-mixing the components. Low initial hematocrit separation runs require smaller sample sizes, especially in the RBC dominant portion of the chamber (the first few samples off the bottom). Perhaps surprisingly, the achieved separation quality recorded for run B19890614a was the second best (SQ=0.4027).



Figure 4-8 Bovine blood separation run B19890605a.

Separation run parameters:blood collected 4 day(s) prior to run;initial hematocrit, HCT=31.50;centrifuge spin speed, RPM=1033 min⁻¹;batch duration, θ =1620 s;separation quality, SQ=0.4072.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.

4.2.4 Varying initial hematocrit

For a fixed centrifuge spin speed and batch duration, better separations favor low initial hematocrit. Less cells to settle take less time to separate. Or looked at from a different angle, higher initial hematocrit increases the influence of hindered settling, retarding the separation.

Run	Graph	Initial hematocrit	Centrifuge spin speed (min ⁻¹)	Batch duration (s)	Separation quality
B19881212a	Figure 4-7	38.8	1161	1560	0.2069
B19890605a	Figure 4-8	31.5	1033	1620	0.4072

Table 4-6 Variable initial hematocrit, separation runs.

4.3 Human blood runs

Having optimized the V-3 centrifuge with bovine blood runs, the "best" operating conditions were selected, and the centrifuge, as part of an NJIT mini-lab, was taken to UMDNJ for human blood runs. Four separation runs were completed. The operating conditions are summarized in Table 4-7.

Run	Graph	Initial hematocrit	Centrifuge spin speed (min ⁻¹)	Batch duration (s)	Separation quality
H19900127a	Figure 4-9	46.3	892	1460	0.7148
H19900127b	Figure 4-10	46.3	892	1340	0.8295
H19900127c	Figure 4-11	46.3	892	780	0.8361
H19900127d	Figure 4-12	46.3	892	630	0.7508

 Table 4-7 Human blood separation runs.

Peak separations such as shown in the accumulated cell-fraction graphs below, are far superior to those experienced with bovine blood runs. The reasons could include rouleaux formation kinetics, Boycott effect, boundary interactions, cell morphology, differences in RBC/WBC densities for human versus bovine blood. It is stunning that human blood separation graphs look more like the hypothesized, ideal separation graph of Figure 4-1 than like any of the bovine blood runs. Run H19900127d, the shortest duration batch has a SQ=0.7508. Run H19900127c, being some what longer batch duration has an increased SQ=0.8361. Run H19900127b, is close to twice the duration of the run C, yet its SQ=0.8295—it has reversed the trend, though only slightly, the separation quality has diminished. Now, run H19900127a, is about 2 minutes longer than run B, but the separation quality has substantially diminished, SQ=0.7148. Why? The RBC packed bed has reached maximum packing factor at approximately θ = 780 seconds (run C). Increases in batch duration have little to no effect on the RBC bed, but it does effect the WBC separation line (check the slope of the WBC line in the graphs): the result is a decrease in SQ after having passed some optimum batch duration between 780 and 1340 seconds.



Figure 4-9 Human blood separation run H19900127a.

Separation run parameters:blood collected 0 day(s) prior to run;initial hematocrit, HCT=46.3;centrifuge spin speed, RPM=892 min⁻¹;batch duration, θ =1460 s;separation quality, SQ=0.7148.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating the

separation: the X=Y line corresponds to zero separation; the packed-RBC line represents ideal RBC separation.



Figure 4-10 Human blood separation run H19900127b.

Separation run parameters:blood collected 0 day(s) prior to run;initial hematocrit, HCT=46.3;centrifuge spin speed, RPM=892 min⁻¹;batch duration, θ =1340 s;separation quality, SQ=0.8295.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.

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Separation run parameters: initial hematocrit, HCT=46.3; batch duration, θ =780 s; The X=X line (decked) and packed PPC line (detted) provide viewal guides for evaluation

The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating the separation: the X=Y line corresponds to zero separation; the packed-RBC line represents ideal RBC separation.





Separation run parameters:blood collected 0 day(s) prior to run;initial hematocrit, HCT=46.3;centrifuge spin speed, RPM=892 min⁻¹;batch duration, θ =630 s;separation quality, SQ=0.7508.The X=Y line (dashed) and packed-RBC line (dotted) provide visual guides for evaluating theseparation: the X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.

Figure 4-13, Figure 4-14, Figure 4-15, and Figure 4-16 show a regressed leastsquares fitted line through the first four sample data points in each of the four human separation runs. The conditions are similar, and the closeness of the initial slopes shows repeatability among the four runs.

Notice that the slopes of the fitted line for runs Ha–Hc are basically the same, but the shortest batch duration run H19900127d has a different slope. The slope of the line is directly attributable to the condition of the RBC packed bed. For a maximum packed bed the RBC line will also be a maximum, increased batch duration will have little to no effect. This is the case for the three longest batch runs: the slope is the same, because the RBC bed has reached maximum packing factor at or around 780 seconds. The increased batch runs can not squeeze the cells in the packed bed any tighter.

Run	Slope	R^2_{\perp}
H19900127a	1.6763	0.9984
H19900127b	1.6945	0.9986
H19900127c	1.6258	0.9989
H19900127d	1.5003	0.9989

 Table 4-8 Regressed line through first four data points.





Separation run parameters: initial hematocrit, HCT=46.3; batch duration, θ =1460 s; slope=1.6763*RBC; blood collected 0 day(s) prior to run; centrifuge spin speed, RPM=892 min⁻¹; separation quality, SQ=0.7148; correlation coefficient, R²=0.9984.





Separation run parameters: initial hematocrit, HCT=46.3; batch duration, θ =1340 s; slope=1.6945*RBC; blood collected 0 day(s) prior to run; centrifuge spin speed, RPM=892 min⁻¹; separation quality, SQ=0.8295. correlation coefficient, R²=0.9986.



Figure 4-15 Initial slope for human blood separation run H19900127c.

Separation run parameters: initial hematocrit, HCT=46.3; batch duration, θ =780 s; slope=1.6258*RBC; blood collected 0 day(s) prior to run; centrifuge spin speed, RPM=892 min⁻¹; separation quality, SQ=0.8361. correlation coefficient, R²=0.9989.



Figure 4-16 Initial slope for human blood separation run H19900127d.

Separation run parameters: initial hematocrit, HCT=46.3; batch duration, θ =630 s; slope=1.5003*RBC; blood collected 0 day(s) prior to run; centrifuge spin speed, RPM=892 min⁻¹; separation quality, SQ=0.7508. correlation coefficient, R²=0.9989.

Additional Separation Runs

The remaining bovine and human blood separations are collected in the Appendix B for the sake of completeness.

4.4 Optimum

Under the influence of gravity or centrifugal force, the formed elements of blood within a container sediment toward the bottom. After adequate time the cells are completely

settled. The cells accumulate at the bottom making layers of cells, from bottom to top: RBCs, WBCs, platelets, plasma.

The cells fall at different rates due to their individual properties: their shape, their density, their size, tendency to flocculate (clumping and rouleaux formation), drag coefficient, concentration of neighboring cells. Between the start of the sedimentation process of a mixture and the eventual end point, the sedimentation process is dynamic. So, if a batch sedimentation is stopped prior to complete settling (as is the case with the present study), the resulting separation may be successful or not as a function of all the independent variables that describe the system.

During the sedimentation process, three dynamic zones are forms in the separation chamber—packed-cell-bed or precipitate zone, constant-velocity settling zone, plasma supernatant zone. At the bottom of the chamber, settling cells come to rest and build up into a packed bed of resting cells. The cells pack down, closer and closer; the bed builds up, higher and higher. Above the bed are all the settling cells falling at their respective constant settling velocity (assuming sedimentation in a constant gravitational field). Just as accumulating cells form a packed bed of cells at the bottom of the chamber, similarly, the cells evacuating from the top of the chamber leave and plasma replaces the abandoned space making a plasma supernate.

While cells are falling at respective settling velocity in the settling zone, the faster cells will separate from the slower cells; the longer they settle the more they separate. But the cells must eventually reach the bottom of the chamber and come to a halt. Here is where competing phenomena come into play and allow the possibility of an optimum. The fastest settling cells separate from the slower settling cells as time progresses; yet,

the same fastest settling cells also reach the bottom of the chamber first followed subsequently by the slower settling cells, as time progresses the separated slower cells approach the arrested, faster settling cells. Hence, the distinct possibility that an optimum set of operating conditions exist and may be found.

		1		
Run	Initial HCT	RPM	Batch duration	SQ
Α	46.3	892	1460	0.7148
В	46.3	892	1340	0.8295
С	46.3	892	780	0.8361
D	46.3	892	630	0.7508

 Table 4-9 Human blood separation quality (SQ) values

Human Blood versus Bovine blood

It is evident that the human blood cell separations far exceeded those of even the best bovine blood cell separations. This is undoubtedly a combination of factors associated with the properties of bovine blood cells compared to human blood cells. The two most likely reasons for human blood's high SQ performance are (1) more extensive humanblood rouleaux formation and (2) larger human RBC.

CHAPTER 5

MATHEMATICAL MODELING

5.1 History

Mathematical simulation of centrifuging blood, whether using a simple model or sophisticated, is a problem of compounded difficulty—a complex fluid yields a complex modeling problem. Fluids do not come much more complicated than blood. The model described below and in Appendix A is presented for the purpose of documentation: a history of the work, as far as it went. The system of partial differential equations generated from this model was unsolvable using a collocation, finite element method solver with options for stiff systems. We do not recommend pursuing the solution of this model further, nor do we recommend attempting alterations in the model itself in search of a solvable system of equations. All evidence suggests that this volume-diffusion model is intractable.

5.2 Basis in Molecular Diffusion

The math model starts with the description of multicomponent fluxes in terms of transport properties in section 18.4 of *Transport Phenomena* (*TP*) by Bird, Stewart, and Lightfoot (1960). The equations presented in this section of *TP* are based on two publications: (1) the article *Fluid Mechanics and the Transport Phenomena* by Bird, Curtiss, and Hirschfelder (1955), and (2) the book *Molecular Theory of Gases and Liquids* by-Hirschfelder, Curtiss, and Bird (1954). In these works expressions for the mass flux, j_i , in a multicomponent system are developed based on the molecular theory

of gases and liquids. Bird, Stewart, and Lightfoot summarize the most general expression of mass flux (*TP* 18.4-7–18.4-11) broken into contributions for ordinary diffusion, $j_i^{(x)}$, pressure diffusion, $j_i^{(p)}$, forced diffusion, $j_i^{(g)}$, and thermal diffusion, $j_i^{(T)}$. They also provide a simplification for a binary system (*TP* 18.4-14, 18.4-15), which was the starting point for the volume-diffusion model (VDM) developed here.

The volume-diffusion model extends the original expressions for mass flux, j_i , and to volume flux, j_i^{\bullet} . The diffusion of RBCs and WBCs in plasma is not the movement of molecules but of blood cells: volume diffusion. As a RBC settles the volume of the cell relocates to its new position and the previous location becomes occupied by plasma equal to the volume of the cell. This is a volumetric process; molar flux and mass flux do not describe the phenomena.

5.3 Volume Diffusion Model

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Equation of Motion

$$-\rho \frac{v_{\theta}^{\bullet 2}}{r} = -\frac{\partial p}{\partial r}$$

Equation of Continuity

$$-\frac{\partial n_a^{\bullet}}{\partial r} - \frac{1}{r} n_a^{\bullet} = \frac{\partial}{\partial t} \phi_a$$

Volume flux with respect to stationary coordinates

$$n_{a}^{\bullet} = \hat{V}_{a}\hat{V}_{b}\left(\frac{\phi_{a}}{\hat{V}_{a}} + \frac{\phi_{b}}{\hat{V}_{b}}\right)\left\{\frac{-c^{2}}{\rho}M_{a}M_{b}D_{ab}\left[\frac{\partial}{\partial r}\left(\frac{\rho_{a}}{M_{a}} + \frac{\rho_{b}}{M_{a}}\right) + \left(\frac{\rho_{a}}{M_{a}} - \frac{\rho_{a}}{M_{a}}M_{a}\right) + \left(\frac{\rho_{a}}{M_{a}} - \frac{\rho_{a}}{M_{a}}M_{a}\right)\right]\right\}$$

Boundary Conditions

IC
$$t = 0$$
, $\phi_a = \phi_{a,0}$
BC1 $r = r_1$, $\phi_a = 0$
BC2 $r = r_2$, $\phi_a = \phi_{a,max}$

The binary model, as expressed here, in terms of time, t, space, r, and density, ρ_i , should be rearranged in terms of one component and with the density replaced by volume fraction of RBCs (or hematocrit), ϕ_a . This may be accomplished by way of these simple substitutions,

$$\rho_a = \frac{\phi_a}{\hat{V}_a},$$
$$\phi_b = 1 - \phi_a.$$

In order to conserve space, the complete volume-diffusion model with all appropriate substitutions is included in the Appendix A.

Notation

- $c = c_a + c_b = 1/(M_a \hat{V}_a + M_b \hat{V}_b) = \text{molar concentration of mixture (scalar), mole}/L^3$;
- D_{ab} = binary diffusivity for system A-B (scalar), L^2/t ;
- M_i = molar mass of species *i* (scalar), M/mole;
- n_i^{\bullet} = volume flux of component *i* with respect to stationary coordinates (vector), $M/L^2 t$;
- ϕ_i = volume fraction of *i* (scalar), L^3/L^3 [Note: for red blood cells, volume fraction equals hematocrit.];
- p = fluid pressure (scalar), M/Lt^2 ;
- r = radial distance in cylindrical coordinate system (scalar), L;
- ρ = fluid density of mixture (scalar), M/L^3 ;
- ρ_i = mass concentration of species *i* (scalar), M/L^3 [Note: mass is relative to mixture volume; this is <u>not</u> a pure-component density.];

- v_{θ}^{\bullet} = volume-average velocity in the θ direction (scalar), L/t [Angular velocity of the spinning centrifuge.];
- \hat{V}_i = pure-component specific volume of *i* (scalar), L^3/M [inverse of pure-component density].

5.4 Solver

The VDM had to be solved by a numerical method due to the complexity of the system of partial differential equations (PDE). A general collocation software package for PDEs developed by Madsen and Sincovec (1979) and published in Transactions of Mathematical Software (ACM-TOMS), algorithm 540, called PDECOL. The PDECOL computer software package is designed to solve coupled systems of nonlinear partial differential equations in one space and one time dimension. (This describes the VDM system.) the package implements finite element collocation methods based on piecewise polynomials for the spatial discretization techniques. The time integration process is then accomplished using the method of lines and standard time stepping algorithms. The code was written in Fortran.

The PDECOL algorithm was unable to converge to a solution for the VDM. Because the original code was written in single precision, we converted the code to double precision hoping that the added precision would allow the program to converge to a solution, but the result was unchanged—no convergence. Several attempts were made to circumvent the convergence problem: varying the convergence criteria, rechecking all physical constants, simplified versions of the VDM.

In the end, it was concluded that the VDM system cannot be solved with PDECOL. It might be solved sometime in the future, by an alternative numerical method.

The investigators suspect that the VDM system may be intractable because of the extreme complexity of the system of equations and the necessity of including parameters representing the molecular weights of RBCs and plasma, which are very large numbers and could contribute to the stiffness of the system and resulting difficulty reaching a solution. It is believed that the volume-diffusion model should be abandoned as a viable mathematical model for simulating the sedimentation of blood cells in plasma.
CHAPTER 6 '

CONCLUSIONS AND RECOMMENDATIONS

Concerning operating parameters (initial hematocrit, centrifuge spin speed, batch duration): even though initial hematocrit is a critical operating parameter, especially with its large influence on rouleaux formation, its practical usefulness as an operating parameter is diminished by the relative difficulty of adjusting its value via dilution. Therefore, while the importance of adjusting the starting hematocrit was initially stressed, it is no longer recommended that initial hematocrit be manipulated by dilutions prior to running V-3.

The difference between a good separation and a bad separation is often just a matter of time. When separations have not reached completion, one may expect better separation with increased batch duration, everything else staying the same. The same is also true for centrifuge spin speed. Faster centrifuge spin speed results in better separation, if separation has not reached completion. This is to be expected given the effect of centrifuge spin speed on the sedimentation rate of RBCs and WBCs—as spin speed increases, sedimentation rate increases, and required batch duration for a given SQ decreases. The experimental operating procedure for batch separations developed in this investigation yields good separations when the values of the operating parameters allow it.

The accumulated cell-fraction graphical technique for displaying separation results developed here is a useful method for compact presentation of and identification of good or bad separations. A separation may be quantified by viewing the maximum

separation between the RBC fraction and WBC fraction curves; alternatively and conveniently, this value may be calculated from the sample cell fractions.

Separation quality (SQ) values have been calculated for the 15 bovine blood separation runs and are summarized in variously sorted tables included in appendix C; a graph of the bovine runs may be viewed in figures G-1 through G-3 (in the Appendix G). In figure G-1 the three lowest SQs are present in the runs with shortest batch duration. Also, for the low-duration runs, the best separations are the low-initial-hematocrit runs. For runs greater than 2500 seconds duration, general separation quality is best, regardless of initial hematocrit or centrifuge spin speed (rpm).

Separation quality (SQ) values have also been calculated for the four human blood runs. Figure G-4 (located in Appendix G) compares SQ with batch duration. The small human blood study was restricted to constant initial hematocrit (46.3%) and constant centrifuge spin speed (892rpm). The graph in figure G-4 clearly shows an optimum between 780 seconds and 1340 seconds duration: the optimum is bounded.

The volume diffusion model does not work. A finite element collocation method numerical solution was attempted for the binary system of RBC and plasma. The solver could not converge to an answer. It may be that another numerical method will be able to solve the volume diffusion model's system of partial differential equations. In lieu of a future solution, the system appears to be intractable. This situation is likely a consequence of the transformation from the original molecular flux terms into volume flux (that describe RBCs in plasma) and the conversion requirement of including molecular weight terms for the RBCs and plasma. The inability to cancel out the many similar terms in the expression for volume flux, because of the molecular weight terms littering the expression, yields a cumbersome system of partial differential equation.

Modeling is useful and important, and another mathematical model should be pursued. The human blood separations were very successful. Expanded studies in this area are warranted and highly recommended. The optimization of chamber shapes should be pursued.

APPENDIX A

VOLUMETRIC-DIFFUSION MODEL AND FLUX TERMS

A.1 Volumetric-Diffusion Model

A.1.1 Equation of Motion

$$-\rho \frac{v_{\theta}^{*^2}}{r} = -\frac{\partial p}{\partial r} \tag{A.1}$$

A.1.2 Equation of Continuity

$$-\frac{\partial n_a^{\bullet}}{\partial r} - \frac{1}{r} n_a^{\bullet} = \frac{\partial}{\partial t} \phi_a \tag{A.2}$$

A.1.3 Boundary Conditions

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A 1-D initial-boundary-value problem requires three boundary conditions; the specific boundary conditions (IC= initial condition, BC= boundary condition) for this problem are the following:

IC
$$t = 0, \quad \phi_a = \phi_{a,0},$$

BC1 $r = r_1, \quad \phi_a = 0,$ (A.3)
BC2 $r = r_2, \quad \phi_a = \phi_{a,\max}.$

A.2 Volume flux and its derivative

Reminder: some helpful and necessary rules of differentiation. These four equations will be used either explicitly or implicitly, as they are rudimentary to the task at hand, especially (A.5).

Product rule

$$\frac{d}{dx}(uv) = u\frac{dv}{dx} + v\frac{du}{dx}$$
(A.4)

Product rule, applied to a ratio

$$\frac{d}{dx}\left(\frac{u}{v}\right) = \frac{v\frac{du}{dx} - u\frac{dv}{dx}}{v^2}$$
(A.1)

Power rule

$$\frac{d}{dx}\left(u^{n}\right) = nu^{n-1}\frac{du}{dx} \tag{A.2}$$

Natural logarithm function

$$\frac{d}{dx}\ln u = \frac{1}{u}\frac{du}{dx} \tag{A.3}$$

A.3 Partial derivative of volume flux with respect to radius

To find the partial derivative of volume flux with respect to radius for a stationary coordinate system, we begin with the volume flux.

$$n_{a}^{\bullet} = \left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)\left\{\frac{-\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)}\right\}$$

$$D_{ab}\left[\frac{\partial}{\partial r}\left(\frac{\phi_{a}M_{b}\hat{V}_{b}}{\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}}\right)+\left(\frac{\phi_{a}M_{b}\hat{V}_{b}}{\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}}-\frac{\phi_{a}\hat{V}_{b}}{\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}}\right)\frac{\partial\ln p}{\partial r}\right]\right\}$$
(A.4)

Where:

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- $c = c_a + c_b = 1/(M_a\hat{V}_a + M_b\hat{V}_b) = \text{molar concentration of}$ mixture (scalar), mole/ L^3 ;
- D_{ab} = binary diffusivity for system A-B (scalar), L^2/t ;
- M_i = molar mass of species *i* (scalar), M/mole;
- n_i^{\bullet} = volume flux of component *i* with respect to stationary coordinates (vector), $M/L^2 t$;
- ϕ_i = volume fraction of *i* (scalar), L^3/L^3 [Note: for red blood cells, volume fraction equals hematocrit.];
- p =fluid pressure (scalar), M/Lt^2 ;
- r = radial distance in cylindrical coordinate system (scalar), L;

- ρ = fluid density of mixture (scalar), M/L^3 ;
- ρ_i = mass concentration of species *i* (scalar), M/L^3 [Note: mass is relative to mixture volume; this is <u>not</u> a purecomponent density.];
- v_{θ}^{\bullet} = volume-average velocity in the θ direction (scalar), L/t[Angular velocity of the spinning centrifuge.];
- \hat{V}_i = pure-component specific volume of *i* (scalar), L^3/M [inverse of pure-component density].

Equation (A.4) is rather large. Rewrite it in a more manageable size by substituting abbreviations for the larger terms in the expression.

$$n_{a}^{\bullet} = a(r) \left\{ \frac{b(r)}{c(r)} D_{ab} \left[\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \frac{\partial \ln p}{\partial r} \right] \right\}$$
(A.5)

Where:

$$a(r) = \phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a}; \qquad b(r) = -\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right)^{2}; \qquad c(r) = \left(M_{a} \hat{V}_{a} M_{b} \hat{V}_{b} \right) \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right); \\ d(r) = \phi_{a} M_{b} \hat{V}_{b};$$

$$e(r) = \phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a; \qquad f(r) = \phi_a \hat{V}_b; \qquad g(r) = \phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a$$

A.3.1 Partial derivative of the volume flux, $\partial n_a^{\bullet} / \partial r$

First, symbolically write the expression using the above alphabetic, inter-equation functions and a convenient shorthand of empty braces and empty brackets to represent the corresponding braced and bracketed terms in (A.5) (which is repeated here).

$$n_{a}^{\bullet} = a(r) \left\{ \frac{b(r)}{c(r)} D_{ab} \left[\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \frac{\partial \ln p}{\partial r} \right] \right\}$$

$$\left\{ \right\} = \left\{ \frac{b(r)}{c(r)} D_{ab} \left[\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \frac{\partial \ln p}{\partial r} \right] \right\}$$

$$\left[\right] = \left[\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \frac{\partial \ln p}{\partial r} \right]$$
(A.6)

Now express the partial derivative of the volume flux, $\partial n_a^* / \partial r$, using the product rule of differentiation. Combining these substitutions yields an abbreviated, yet exploded, skeleton of the partial derivative of the volume flux; the result illustrates the order of differentiation.

$$n_{a}^{*} = a(r) \left\{ \frac{b(r)}{c(r)} D_{ab} \left[\right] \right\}$$

$$\frac{\partial n_{a}^{*}}{\partial r} = a(r) \frac{\partial \left\{ \right\}}{\partial r} + \left\{ \right\} \frac{\partial}{\partial r} (a(r))$$

$$\frac{\partial \left\{ \right\}}{\partial r} = \frac{b(r)}{c(r)} \frac{\partial \left[\right]}{\partial r} + \left[\right] \frac{\partial}{\partial r} \left(\frac{b(r)}{c(r)} \right)$$

$$\frac{\partial \left[\right]}{\partial r} = \frac{\partial}{\partial r} \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \frac{\partial}{\partial r} \left(\frac{\partial \ln p}{\partial r} \right) + \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) - \frac{\partial}{\partial r} \left(\frac{f(r)}{g(r)} \right) \right) \frac{\partial \ln p}{\partial r}$$
(A.7)

Find the more conventional, but still abbreviated, representation of the partial derivative by collapsing the skeletal set of equations for volume flux, (A.7) and substituting the appropriate empty-braced or empty-bracketed term from (A.6).

$$\frac{\partial n_{a}^{\bullet}}{\partial r} = a(r)\frac{b(r)}{c(r)}\frac{\partial}{\partial r}\left(\frac{\partial}{\partial r}\left(\frac{d(r)}{e(r)}\right)\right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)}\right)\frac{\partial}{\partial r}\left(\frac{\partial\ln p}{\partial r}\right) + \left(\frac{\partial}{\partial r}\left(\frac{d(r)}{e(r)}\right) - \frac{\partial}{\partial r}\left(\frac{f(r)}{g(r)}\right)\right)\frac{\partial\ln p}{\partial r} + \left[\frac{\partial}{\partial r}\left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)}\right)\frac{\partial\ln p}{\partial r}\right]\frac{\partial}{\partial r}\left(\frac{b(r)}{c(r)}\right) + \left\{\frac{b(r)}{c(r)}D_{ab}\left[\frac{\partial}{\partial r}\left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)}\right)\frac{\partial\ln p}{\partial r}\right]\right\}\frac{\partial}{\partial r}(a(r))$$
(A.8)

There are, however, unknown terms in the shorthand partial derivative, (A.8); all the differential terms are unknown; all the terms involving pressure, p, are unknown. So before proceeding, equivalent expressions are required. To fulfill that requirement all the

differential terms must be derived along with any expressions that involve pressure, p. We already know the expressions for the algebraic functions or can find them from (A.5); for convenience they are repeated here.

$$a(r) = \phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a}$$

$$\frac{b(r)}{c(r)} = \frac{-\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right)^{2}}{\left(M_{a} \hat{V}_{a} M_{b} \hat{V}_{b} \right) \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right)}$$

$$\frac{d(r)}{e(r)} = \frac{\phi_{a} M_{b} \hat{V}_{b}}{\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a}}$$

$$\frac{f(r)}{g(r)} = \frac{\phi_{a} \hat{V}_{b}}{\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a}}$$
(A.9)

A.3.2 Partial derivative of the function a(r)

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Now we begin finding the unknown terms to fill in the skeleton, (A.7). First, find the partial derivative of a(r)—recall the expression of the function from (A.9).

$$\frac{\partial}{\partial r} (a(r)) = \frac{\partial}{\partial r} \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a \right)$$
$$\frac{\partial a(r)}{\partial r} = \frac{\partial \phi_a}{\partial r} \left(\hat{V}_b - \hat{V}_a \right)$$
(A.10)

A.3.3 Partial derivative of the expression b(r)/c(r)

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Next, derive the expression for the partial derivative of the ratio b(r)/c(r) as defined in (A.9).

$$\frac{\partial}{\partial r} \left(\frac{b(r)}{c(r)} \right) = \frac{\partial}{\partial r} \left(\frac{-\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^2}{\left(M_a \hat{V}_a M_b \hat{V}_b \right) \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a \right)} \right) = \frac{\text{top}}{\text{bottom}}$$

This derivation follows the product rule for a ratio, (A.1). As the denominator (bottom) is simply the square of the original denominator, we will concentrate on the numerator (top).

Collect common terms of the numerator, (A.11).

Concentrating on the expression within the brackets in (A.12); expand it;

$$\left[-2\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)\right)-2\hat{V}_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)\right)+M_{a}\hat{V}_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)\right]$$
(A.13)

then cancel and simplify.

,

$$\left[-\left(M_b\hat{V}_b - M_a\hat{V}_a\right)\left(\phi_a\left(\hat{V}_b - \hat{V}_a\right) + 2\hat{V}_a\right) + M_a\hat{V}_a\left(\hat{V}_b - \hat{V}_a\right)\right]$$
(A.14)

Put the bracketed expression back into the numerator (top).

$$\frac{\partial}{\partial r} \left(\frac{b(r)}{c(r)} \right) = \frac{\frac{\partial \phi_a}{\partial r} \left(M_a \hat{V}_a M_b \hat{V}_b \right) \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right) \left[- \left(M_b \hat{V}_b - M_a \hat{V}_a \right) \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + 2\hat{V}_a \right) + M_a \hat{V}_a \left(\hat{V}_b - \hat{V}_a \right) \right]}{\left(M_a \hat{V}_a M_b \hat{V}_b \right)^2 \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a \right)^2}$$
(A.15)

The term, $(M_a \hat{V}_a M_b \hat{V}_b)$, which appears in both the numerator and the denominator may be canceled out. The result is the partial

derivative.

$$\frac{\partial}{\partial r} \left(\frac{b(r)}{c(r)} \right) = \frac{\frac{\partial \phi_a}{\partial r} \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right) \left[- \left(M_b \hat{V}_b - M_a \hat{V}_a \right) \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + 2 \hat{V}_a \right) + M_a \hat{V}_a \left(\hat{V}_b - \hat{V}_a \right) \right]}{\left(M_a \hat{V}_a M_b \hat{V}_b \right) \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a \right)^2}$$
(A.16)

A.3.4 Partial derivative of d(r)/e(r)

Derive the partial derivative of the ratio, d(r)/e(r), which is defined in (A.9).

$$\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) = \frac{\partial}{\partial r} \left(\frac{\phi_a M_b \hat{V}_b}{\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a} \right) = \frac{\mathrm{top}}{\mathrm{bottom}} = \frac{\mathrm{top}}{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^2}$$
(A.17)

Concentrating on the numerator (top),

$$\operatorname{top} = \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a\right) + M_a \hat{V}_a\right) \left(M_b \hat{V}_b\right) \frac{\partial \phi_a}{\partial r} - \phi_a M_b \hat{V}_b \left((1) \left(M_b \hat{V}_b - M_a \hat{V}_a\right)\right) \frac{\partial \phi_a}{\partial r}$$
(A.18)

simplify

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$$top = \frac{\partial \phi_a}{\partial r} \left(M_b \hat{V}_b \right) \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a - \phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) \right)$$
(A.19)

and recombine numerator (top) and denominator (bottom).

$$\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) = \frac{\frac{\partial \phi_a}{\partial r} \left(M_a \hat{V}_a M_b \hat{V}_b \right)}{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^2}$$
(A.20)

A.3.5 Partial of the partial derivative of d(r)/e(r)

Continuing with the ratio d(r)/e(r), we derive the second partial derivative with respect to the radius (the partial derivative of the partial derivative). Start from where we left off with the first partial derivative, (A.20).

- -

$$\frac{\partial}{\partial r} \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) \right) = \frac{\partial}{\partial r} \left(\frac{\frac{\partial \phi_a}{\partial r} \left(M_a \hat{V}_a M_b \hat{V}_b \right)}{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^2} \right) = \frac{\mathrm{top}}{\mathrm{bottom}} = \frac{\mathrm{top}}{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^4}$$
(A.21)

Focus on the numerator (top).

$$top = \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a\right) + M_a \hat{V}_a\right)^2 \frac{\partial^2 \phi_a}{\partial r^2} \left(M_a \hat{V}_a M_b \hat{V}_b\right) - \frac{\partial \phi_a}{\partial r} \left(M_a \hat{V}_a M_b \hat{V}_b\right) 2 \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a\right) + M_a \hat{V}_a\right) \frac{\partial \phi_a}{\partial r} \left(M_b \hat{V}_b - M_a \hat{V}_a\right) + M_a \hat{V}_a\right) + M_a \hat{V}_a \hat{V}_b \hat{V}_b + M_a \hat{V}_b \hat{V$$

Collect common terms.

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$$top = \left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a\right) + M_a \hat{V}_a\right) \left(M_a \hat{V}_a M_b \hat{V}_b\right) \left(\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a\right) + M_a \hat{V}_a\right) \frac{\partial^2 \phi_a}{\partial r^2} - 2\left(M_b \hat{V}_b - M_a \hat{V}_a\right) \left(\frac{\partial \phi_a}{\partial r}\right)^2\right) \tag{A.23}$$

Substitute the new expression for the numerator (A.23) into (A.21),

$$\frac{\partial}{\partial r} \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) \right) = \frac{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right) \left(M_a \hat{V}_a M_b \hat{V}_b \right) \left(\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right) \frac{\partial^2 \phi_a}{\partial r^2} - 2 \left(M_b \hat{V}_b - M_a \hat{V}_a \right) \left(\frac{\partial \phi_a}{\partial r} \right)^2 \right)}{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^4}$$
(A.24)

and simplify the result.

$$\frac{\partial}{\partial r} \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) \right) = \frac{\left(M_a \hat{V}_a M_b \hat{V}_b \right) \left(\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right) \frac{\partial^2 \phi_a}{\partial r^2} - 2 \left(M_b \hat{V}_b - M_a \hat{V}_a \right) \left(\frac{\partial \phi_a}{\partial r} \right)^2 \right)}{\left(\phi_a \left(M_b \hat{V}_b - M_a \hat{V}_a \right) + M_a \hat{V}_a \right)^3}$$
(A.25)

A.3.6 Partial derivative of f(r)/g(r)

Derive the partial derivative of the ratio f(r)/g(r).

$$\frac{\partial}{\partial r} \left(\frac{f(r)}{g(r)} \right) = \frac{\partial}{\partial r} \left(\frac{\phi_a \hat{V}_b}{\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a} \right) = \frac{\text{top}}{\text{bottom}} = \frac{\text{top}}{\left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a \right)^2}$$
(A.26)

As has been the pattern, focus on the numerator (top),

$$top = \left(\phi_a \left(\hat{V}_b - \hat{V}_a\right) + \hat{V}_a\right) \hat{V}_b \frac{\partial \phi_a}{\partial r} - \phi_a \hat{V}_b \left(\hat{V}_b - \hat{V}_a\right) \frac{\partial \phi_a}{\partial r}$$
(A.27)

collect the common terms

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$$top = \hat{V}_b \frac{\partial \phi_a}{\partial r} \left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a - \phi_a \left(\hat{V}_b - \hat{V}_a \right) \right)$$
(A.28)

and simplify.

$$top = \hat{V}_a \hat{V}_b \frac{\partial \phi_a}{\partial r} \tag{A.29}$$

Substituting the new expression for the numerator (A.29) into (A.26).

$$\frac{\partial}{\partial r} \left(\frac{f(r)}{g(r)} \right) = \frac{\hat{V}_a \hat{V}_b \frac{\partial \phi_a}{\partial r}}{\left(\phi_a \left(\hat{V}_b - \hat{V}_a \right) + \hat{V}_a \right)^2}$$
(A.30)

A.3.7 Handling the pressure term, $\partial (lnp)/\partial r$

The volume flux contains a deferential pressure term that must be replaced, because pressure is an independent variable, which is a function of space and time. Begin with the continuity equation.

$$\frac{\partial p}{\partial r} = \frac{\rho v_{\theta}^{*2}}{r}$$
(A.31)

Separate the variables (in differential form).

$$dp = \rho v_{\theta}^{\bullet^2} \frac{dr}{r} \tag{A.32}$$

Express the differential equation, (A.32), in integral form—integrating the left-hand-side from p_{min} (or p_{atm}) to p and the right-hand-side from r_{min} to r. Here p_{min} is the minimum pressure, which happens to be atmospheric pressure (p_{atm}). The variable r_{min} is the corresponding radius at which the minimum pressure occurs.

$$\int_{p_{\min}=p_{alm}}^{p} dp = \rho v_{\theta}^{\bullet^{2}} \int_{r_{\min}}^{r} \frac{dr}{r} = \rho v_{\theta}^{\bullet^{2}} \int_{r_{\min}}^{r} d\left(\ln r\right)$$
(A.33)

After integrating (A.33) over the indicated bounds,

$$p - p_{\text{atm}} = \rho v_{\theta}^{\bullet^2} \left(\ln r - \ln r_{\min} \right)$$
(A.34)

move p_{atm} to the other side of the equation and replacing ρ with its equivalent, $(\hat{V}_a + \hat{V}_b)^{-1}$.

$$p = p_{\text{atm}} + \frac{v_{\theta}^{\star^2} \left(\ln r - \ln r_{\min} \right)}{\left(\hat{V}_a + \hat{V}_b \right)}$$
(A.35)

This equation, (A.35), is required for a substitution (below) to eliminate pressure, p.

Starting again with the equation of continuity, (A.31), divide both sides by pressure.

$$\frac{\frac{\partial p}{p}}{\partial r} = \frac{\rho v_{\theta}^{\bullet^2}}{pr}$$
(A.36)

Substitute $\rho = (\hat{V}_a + \hat{V}_b)^{-1}$ and $\frac{\partial p}{p} = \partial (\ln p)$.

$$\frac{\partial \left(\ln p\right)}{\partial r} = \frac{v_{\theta}^{*^2}}{pr\left(\hat{V}_a + \hat{V}_b\right)}$$
(A.37)

Substitute (A.35) into (A.37),

,

$$\frac{\partial (\ln p)}{\partial r} = \frac{v_{\theta}^{*^2}}{r(\hat{V}_a + \hat{V}_b) \left(p_{\text{atm}} + \frac{v_{\theta}^{*^2} (\ln r - \ln r_{\min})}{(\hat{V}_a + \hat{V}_b)} \right)}$$
(A.38)

and rearrange; this yields the partial derivative of the natural logarithm of pressure with respect to the radius.

$$\frac{\partial(\ln p)}{\partial r} = \frac{1}{r\left(\frac{p_{\text{atm}}}{v_{\theta}^{*2}}\left(\hat{V}_{a} + \hat{V}_{b}\right) + \left(\ln r - \ln r_{\min}\right)\right)}$$
(A.39)

A.3.8 Second partial derivative of the pressure term

Begin with (A.39)

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$$\frac{\partial}{\partial r} \left(\frac{\partial (\ln p)}{\partial r} \right) = \frac{\partial}{\partial r} \left(\frac{1}{r \left(\frac{p_{\text{atm}}}{v_{\theta}^{*2}} \left(\hat{V}_{a} + \hat{V}_{b} \right) + \left(\ln r - \ln r_{\min} \right) \right)} \right)$$
(A.40)

Differentiate, using the product rule, applied to a ratio.

$$\frac{\partial}{\partial r} \left(\frac{\partial (\ln p)}{\partial r} \right) = \frac{-1}{r^2 \left(\frac{p_{\text{atm}}}{v_{\theta}^{*2}} \left(\hat{V}_a + \hat{V}_b \right) + (\ln r - \ln r_{\min}) \right)} + \frac{-1}{r \left(\frac{p_{\text{atm}}}{v_{\theta}^{*2}} \left(\hat{V}_a + \hat{V}_b \right) + (\ln r - \ln r_{\min}) \right)^2 r}$$
(A.41)

Rearrange (A.41). This is the partial derivative of the partial derivative of the pressure term in the volume flux.

$$\frac{\partial}{\partial r} \left(\frac{\partial (\ln p)}{\partial r} \right) = \frac{-1}{r \left(\frac{p_{\text{atm}}}{v_{\theta}^{*^{2}}} \left(\hat{V}_{a} + \hat{V}_{b} \right) + (\ln r - \ln r_{\text{min}}) \right)} \left(\frac{1}{r} + \frac{1}{r \left(\frac{p_{\text{atm}}}{v_{\theta}^{*^{2}}} \left(\hat{V}_{a} + \hat{V}_{b} \right) + (\ln r - \ln r_{\text{min}}) \right)} \right) = \frac{-\partial (\ln p)}{\partial r} \left(\frac{1}{r} + \frac{\partial (\ln p)}{\partial r} \right) \quad (A.42)$$

A.3.9 Returning to the partial derivative of the volume flux, $\partial n_a^{\bullet} / \partial r$

This is a reminder of the shape of the partial derivative of the volume flux in skeletal form. The skeleton uses the same parentheses, bracket-, and brace-structure as the filled-in partial derivative (below).

$$\frac{\partial n_{a}^{*}}{\partial r} = (a(r)) \left(\frac{b(r)}{c(r)} \right) \left(\frac{\partial}{\partial r} \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) \right) \right) \\
+ \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \left(\frac{\partial}{\partial r} \left(\frac{\partial \ln p}{\partial r} \right) \right) \\
+ \left(\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) - \frac{\partial}{\partial r} \left(\frac{f(r)}{g(r)} \right) \right) \left(\frac{\partial \ln p}{\partial r} \right) \\
+ \left[\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \left(\frac{\partial \ln p}{\partial r} \right) \right] \left(\frac{\partial}{\partial r} \left(\frac{b(r)}{c(r)} \right) \right) \\
+ \left\{ \left(\frac{b(r)}{c(r)} \right) D_{ab} \left[\frac{\partial}{\partial r} \left(\frac{d(r)}{e(r)} \right) + \left(\frac{d(r)}{e(r)} - \frac{f(r)}{g(r)} \right) \left(\frac{\partial \ln p}{\partial r} \right) \right] \right\} \left(\frac{\partial}{\partial r} (a(r)) \right)$$

Having derived all the terms of the partial derivative of the volume flux, combine them for the completed expression. After some simplification we arrive at the following equation.

$$\begin{split} \frac{\partial n_{\sigma}^{*}}{\partial r} &= \frac{\partial^{2} \phi_{\sigma}}{\partial r^{2}} - \left(\frac{2\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{a} \hat{V}_{s}}{\left(\phi_{s} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{a} \hat{V}_{s}}\right)} \right) \left(\frac{\partial \phi_{s}}{\partial r} \right)^{2} \\ &+ \left(\frac{\phi_{a} M_{s} \hat{V}_{s}}{\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} - \frac{\phi_{s} \hat{V}_{s}}{\phi_{s} \left(\hat{V}_{s} - \hat{V}_{s}\right) + \hat{V}_{s}} \right)} \right) \left(\frac{-1}{r^{2} \left(\frac{P_{am}}{v_{\sigma}^{2}} \left(\hat{V}_{s} + \hat{V}_{s}\right) + (\ln r - \ln r_{\min}) \right)} \right)} \right) + \left(\frac{\partial \phi_{a}}{\partial r} \right)^{2} \left(\frac{M_{s} \hat{V}_{s} M_{s} \hat{V}_{s}}{\left(\phi_{s} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{a} \hat{V}_{s}} \right)^{2} - \frac{\hat{V}_{s} \hat{V}_{s}}{\left(\phi_{s} \left(\hat{V}_{s} - \hat{V}_{s}\right) + \hat{V}_{s}} \right)^{2}} \right) \left(\frac{1}{\left(\frac{P_{am}}{\left(\hat{V}_{s} + \hat{V}_{s}\right) + (\ln r - \ln r_{\min})} \right)} \right)} \right) \\ &+ \left[\left(\frac{\partial \phi_{a}}{\partial r} \right) \left(\frac{\left(M_{s} \hat{V}_{s} M_{s} \hat{V}_{s}\right) + M_{a} \hat{V}_{s}}{\left(\phi_{s} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right)^{2}} \right) + \left(\frac{\phi_{s} M_{s} \hat{V}_{s}}{\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right)^{2} - \frac{\hat{V}_{s} \hat{V}_{s}}{\left(\phi_{s} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + (\ln r - \ln r_{\min})} \right)} \right) \right] \\ &+ \left[\left(\frac{\partial \phi_{a}}}{\left(\partial r} \right) \left(\frac{\left(M_{s} \hat{V}_{s} M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right)^{2} \right)^{2} + \left(\frac{\phi_{s} M_{s} \hat{V}_{s}}{\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \left(- \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \left(\frac{1}{r \left(\frac{P_{am}}{V_{s}^{2}} \left(\hat{V}_{s} - \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \right) \right) \right) \right] \\ &- \left(\frac{\partial \phi_{a}}{\left(\partial r} \right) \left(\frac{\left(M_{s} \hat{V}_{s} M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \left(- \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \left(- \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \right) \right) \right) \\ &- \left(\frac{\partial \phi_{a}}{\left(\partial r} N_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}} \right) + M_{s} \hat{V}_{s}} \right) \left(- \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \left(\frac{\partial \phi_{s}}{\left(\partial r} \hat{V}_{s} - \hat{V}_{s} \right) + M_{s} \hat{V}_{s}} \right) \right) \right) \\ \\ &+ \left(\frac{\partial \phi_{a}}{\left(\partial r} M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}} \right) + M_{s} \hat{V}_{s}} \right) \left(\frac{\partial \phi_{a}}{\left(\partial r} \left(\hat{V}_{s} - \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \right) \left(\frac{\partial \phi_{s}}{\left(\partial r} \hat{V}_{$$

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Rewrite the partial derivative of volume flux, replacing many of the algebraic coefficients with representative functions (defined below).

$$\frac{\partial n_a^{*}}{\partial r} = \frac{\partial^2 \phi_a}{\partial r^2} - f_1(\phi_a) \left(\frac{\partial \phi_a}{\partial r} \right)^2 + f_2(\phi_a) \left(f_3(\ln r) \left(\frac{1}{r} + f_3(\ln r) \right) \right) + \left(\frac{\partial \phi_a}{\partial r} \right) \left(f_4(\phi_a) - f_5(\phi_a) \right) \left(f_3(\ln r) \right) + \left[\left(\frac{\partial \phi_a}{\partial r} \right) f_4(\phi_a) + f_2(\phi_a) \left(f_3(\ln r) \right) \right] + \left[\left(\frac{\partial \phi_a}{\partial r} \right) f_6(\phi_a) + \left\{ f_7(\phi_a) D_{ab} \left[\left(\frac{\partial \phi_a}{\partial r} \right) f_4(\phi_a) + f_2(\phi_a) \left(f_3(\ln r) \right) \right] \right\} \left(\frac{\partial \phi_a}{\partial r} \right) \left(\hat{V}_b - \hat{V}_a \right) + f_2(\phi_a) \left(f_3(\ln r) \right) \right] \right\} \left(\frac{\partial \phi_a}{\partial r} \right) \left(\hat{V}_b - \hat{V}_a \right)$$

Where (note that in the list f_7 and f_6 are out of order, because of the size of f_6):

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$$f_1(\phi_a) = \left(\frac{2\left(M_b\hat{V}_b - M_a\hat{V}_a\right)}{\left(\phi_a\left(M_b\hat{V}_b - M_a\hat{V}_a\right) + M_a\hat{V}_a\right) + M_a\hat{V}_a\right)}\right)$$

$$f_2(\phi_a) = \left(\frac{\phi_aM_b\hat{V}_b}{\phi_a\left(M_b\hat{V}_b - M_a\hat{V}_a\right) + M_a\hat{V}_a} - \frac{\phi_a\hat{V}_b}{\phi_a\left(\hat{V}_b - \hat{V}_a\right) + \hat{V}_a}\right)$$

$$f_{3}(\ln r) = \frac{1}{r\left(\frac{P_{atm}}{v_{\theta}^{*2}}(\hat{V}_{a} + \hat{V}_{b}) + (\ln r - \ln r_{min})\right)} = \frac{\partial(\ln p)}{\partial r} \qquad f_{5}(\phi_{a}) = \frac{\hat{V}_{a}\hat{V}_{b}}{\left(\phi_{a}(\hat{V}_{b} - \hat{V}_{a}) + \hat{V}_{a}\right)^{2}} \\ f_{4}(\phi_{a}) = \frac{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)}{\left(\phi_{a}\left(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a}\right) + M_{a}\hat{V}_{a}\right)^{2}} \qquad f_{7}(\phi_{a}) = \left(\frac{-\left(\phi_{a}\left(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a}\right) + M_{a}\hat{V}_{a}\right)^{2}}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)\left(\phi_{a}\left(\hat{V}_{b} - \hat{V}_{a}\right) + 2\hat{V}_{a}\right) + M_{a}\hat{V}_{a}\left(\hat{V}_{b} - \hat{V}_{a}\right) + \hat{V}_{a}\right)}\right) \\ f_{6}(\phi_{a}) = \left(\frac{\left(\phi_{a}\left(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a}\right) + M_{a}\hat{V}_{a}\right)\left(-\left(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a}\right)\left(\phi_{a}\left(\hat{V}_{b} - \hat{V}_{a}\right) + 2\hat{V}_{a}\right) + M_{a}\hat{V}_{a}\left(\hat{V}_{b} - \hat{V}_{a}\right)\right)}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)\left(\phi_{a}\left(\hat{V}_{b} - \hat{V}_{a}\right) + \hat{V}_{a}\right)^{2}}\right)}\right)$$

Rearrange (A.45) for readability.

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$$\frac{\partial n_a^*}{\partial r} = \frac{\partial^2 \phi_a}{\partial r^2} - f_1(\phi_a) \left(\frac{\partial \phi_a}{\partial r} \right)^2
+ f_2(\phi_a) f_3(\ln r) \left(\frac{1}{r} + f_3(\ln r) \right)
+ \left(\frac{\partial \phi_a}{\partial r} \right) \left(f_4(\phi_a) - f_5(\phi_a) \right) \left(f_3(\ln r) \right)
+ \left[\left(\frac{\partial \phi_a}{\partial r} \right) f_4(\phi_a) + f_2(\phi_a) \left(f_3(\ln r) \right) \right] \left(\frac{\partial \phi_a}{\partial r} \right) f_6(\phi_a)
+ \left\{ f_7(\phi_a) D_{ab} \left[\left(\frac{\partial \phi_a}{\partial r} \right) f_4(\phi_a) + f_2(\phi_a) \left(f_3(\ln r) \right) \right] \right\} \left(\frac{\partial \phi_a}{\partial r} \right) \left(\hat{V}_b - \hat{V}_a \right)$$
(A.46)

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Collect common differential terms.

$$\frac{\partial n_a^{\bullet}}{\partial r} = \frac{\partial^2 \phi_a}{\partial r^2} + \left(\frac{\partial \phi_a}{\partial r}\right)^2 \left(-f_1(\phi_a) + f_4(\phi_a) f_6(\phi_a) + D_{ab}\left(\hat{V}_b - \hat{V}_a\right) f_3(\ln r) f_4(\phi_a) f_7(\phi_a)\right) + \left(\frac{\partial \phi_a}{\partial r}\right) \left[f_3(\ln r) \left(f_4(\phi_a) - f_5(\phi_a) + f_2(\phi_a) f_6(\phi_a) + D_{ab}\left(\hat{V}_b - \hat{V}_a\right) f_2(\phi_a) f_7(\phi_a)\right)\right] + f_2(\phi_a) f_3(\ln r) \left(\frac{1}{r} + f_3(\ln r)\right)$$
(A.47)

Focus on coefficients. First look at the coefficient of the non-linear term, $(\partial \phi_a / \partial r)^2$.

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$$\left(-f_{1}(\phi_{a})+f_{4}(\phi_{a})f_{6}(\phi_{a})+D_{ab}(\hat{V}_{b}-\hat{V}_{a})f_{3}(\ln r)f_{4}(\phi_{a})f_{7}(\phi_{a})\right) = -\left(\frac{2\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)}{\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)}\right) + \left(\frac{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)}{\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}\right) \left(\frac{\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)\left(-\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+2\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)\right)}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)^{2}}\right) \left(A.48) + D_{ab}\left(\hat{V}_{b}-\hat{V}_{a}\right)\left(\frac{1}{r\left(\frac{P_{aim}}{V_{a}^{2}}\left(\hat{V}_{a}+\hat{V}_{b}\right)+\left(\ln r-\ln r_{min}\right)\right)}\right)\left(\frac{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)-M_{a}\hat{V}_{a}^{2}}{\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}\right)\left(\frac{-\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}{M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)^{2}}\right) \right)} \right)$$

Cancel and simplify. This is the coefficient of the non-linear term, $(\partial \phi_a / \partial r)^2$.

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$$\left(-f_{1}(\phi_{a}) + f_{4}(\phi_{a}) f_{6}(\phi_{a}) + D_{ab}(\hat{V}_{b} - \hat{V}_{a}) f_{3}(\ln r) f_{4}(\phi_{a}) f_{7}(\phi_{a}) \right) = - \left(\frac{2(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a})}{(\phi_{a}(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a}) + M_{a}\hat{V}_{a})} \right) + \left(\frac{(-(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a})(\phi_{a}(\hat{V}_{b} - \hat{V}_{a}) + 2\hat{V}_{a}) + M_{a}\hat{V}_{a}(\hat{V}_{b} - \hat{V}_{a}))}{(\phi_{a}(M_{b}\hat{V}_{b} - M_{a}\hat{V}_{a}) + M_{a}\hat{V}_{a})(\phi_{a}(\hat{V}_{b} - \hat{V}_{a}) + \hat{V}_{a})^{2}} \right)$$

$$+ D_{ab}(\hat{V}_{b} - \hat{V}_{a}) \left(\frac{1}{r\left(\frac{p_{atm}}{v_{b}^{*2}}(\hat{V}_{a} + \hat{V}_{b}) + (\ln r - \ln r_{\min})\right)} \right) \left(\frac{-1}{(\phi_{a}(\hat{V}_{b} - \hat{V}_{a}) + \hat{V}_{a})} \right)$$

$$(A.49)$$

Second, look at the coefficient of the first partial derivative, $(\partial \phi_a / \partial r)$.

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$$\begin{split} & \left[f_{3} \left(\ln r \right) \left(f_{4} \left(\phi_{a} \right) - f_{5} \left(\phi_{a} \right) + f_{2} \left(\phi_{a} \right) f_{6} \left(\phi_{a} \right) + \mathcal{D}_{ab} \left(\hat{V}_{b} - \hat{V}_{a} \right) f_{2} \left(\phi_{a} \right) f_{7} \left(\phi_{a} \right) \right) \right] = \\ & \left[\left(\frac{1}{r \left(\frac{P_{atm}}{v_{b}^{*2}} \left(\hat{V}_{a} + \hat{V}_{b} \right) + \left(\ln r - \ln r_{min} \right) \right)} \right) \right] \left(\left(\frac{\left(M_{a} \hat{V}_{a} M_{b} \hat{V}_{b} \right)}{\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right)^{2}} \right) - \left(\frac{\hat{V}_{a} \hat{V}_{b}}{\left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right)^{2}} \right) \right) \\ & + \left(\frac{\phi_{a} M_{b} \hat{V}_{b}}{\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a}} - \frac{\phi_{a} \hat{V}_{b}}{\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + 2 \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) \right) \right) \\ & \left(\frac{\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right) \left(- \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + 2 \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) \right) \right) \\ & \left(\frac{\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right) \left(- \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + 2 \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) \right) \right) \\ & + \mathcal{D}_{ab} \left(\hat{V}_{b} - \hat{V}_{a} \right) \left(\frac{\phi_{a} M_{b} \hat{V}_{b}}{\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a}} - \frac{\phi_{a} \hat{V}_{b}}{\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a}} \right) \left(\frac{-\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right)^{2}}{M_{a} \hat{V}_{a} M_{b} \hat{V}_{b} \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right)^{2}} \right) \right) \right)$$

$$(A.50)$$

Collect common terms and simplify. The coefficient of the first partial derivative is.

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$$\begin{aligned} \left[f_{3}(\ln r) \left(f_{4}(\phi_{a}) - f_{5}(\phi_{a}) + f_{2}(\phi_{a}) f_{6}(\phi_{a}) + D_{ab} \left(\hat{V}_{b} - \hat{V}_{a} \right) f_{2}(\phi_{a}) f_{7}(\phi_{a}) \right) \right] = \\ \left(\frac{1}{r \left(\frac{P_{arm}}{V_{o}^{2}} \left(\hat{V}_{a} + \hat{V}_{b} \right) + \left(\ln r - \ln r_{min} \right) \right)} \right) \left(\left(\frac{\left(M_{a} \hat{V}_{a} M_{b} \hat{V}_{b} \right)}{\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right)^{2}} \right) - \left(\frac{\hat{V}_{a} \hat{V}_{b}}{\left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right)^{2}} \right) \right) \\ + \left(\frac{-\left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right)}{\left(M_{a} \hat{V}_{a} M_{b} \hat{V}_{b} \right) \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right) \right)} \right) \left(\frac{\phi_{a} M_{b} \hat{V}_{b}}{\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a}} - \frac{\phi_{a} \hat{V}_{b}}{\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a}} \right)} \\ \left(\frac{\left(\left(\left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) \left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + 2 \hat{V}_{a} \right) - M_{a} \hat{V}_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) \right)}{\left(\phi_{a} \left(\hat{V}_{b} - \hat{V}_{a} \right) + \hat{V}_{a} \right)} + D_{ab} \left(\hat{V}_{b} - \hat{V}_{a} \right) \left(\phi_{a} \left(M_{b} \hat{V}_{b} - M_{a} \hat{V}_{a} \right) + M_{a} \hat{V}_{a} \right) \right) \right) \right) \end{aligned}$$

Having made all the above substitutions, here is the partial derivative of the volume flux. Arranged according to the magnitude of the volume fraction term.

$$\begin{split} \frac{\partial \sigma_{s}^{*}}{\partial r} &= \frac{\partial^{2} \phi_{s}}{\partial r^{2}} \\ &+ \left(\frac{\partial \phi_{s}}{\partial r} \right)^{2} \left[- \left(\frac{2\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}^{*}\right)}{\left(\phi_{s} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}^{*}\right) + M_{s} \hat{V}_{s}^{*}\right)} \right) \\ &+ \left(\frac{\partial \phi_{s}}{\partial r} \right)^{2} \left[+ \left(\frac{\left(-\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}^{*}\right) \left(\phi_{s}^{*} \left(\hat{V}_{s} - \hat{V}_{s}\right) + 2\hat{V}_{s}^{*}\right) + M_{s} \hat{V}_{s}^{*} \left(\hat{V}_{s} - \hat{V}_{s}^{*}\right)} \right) \\ &+ \left(\frac{\partial \phi_{s}}{\partial r} \right)^{2} \left[+ \left(\frac{1}{V_{a} \delta} \left(\hat{V}_{s} - \hat{V}_{s}\right) + M_{s} \hat{V}_{s}^{*}\right) \left(\phi_{s}^{*} \left(\hat{V}_{s} - \hat{V}_{s}\right) + 2\hat{V}_{s}^{*}\right) + M_{s} \hat{V}_{s}^{*} \left(\hat{V}_{s} - \hat{V}_{s}^{*}\right)} \right) \\ &+ \left(\frac{\partial \phi_{s}}{\partial r} \right)^{2} \left[- \frac{1}{\left(\frac{1}{r \left(\frac{P_{am}}{V_{s}} \left(\hat{V}_{s} + \hat{V}_{s}\right) + \left(\ln r - \ln r_{m}\right)}{r \left(\hat{V}_{a}^{*} \left(\hat{V}_{s} + \hat{V}_{s}\right) + \left(\ln r - \ln r_{m}\right)} \right)} \right) \left(\frac{\left(M_{s} \hat{V}_{s} M_{s} \hat{V}_{s}\right)}{\left(\phi_{s} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}^{*}\right)} \right) \\ &+ \left(\frac{\partial \phi_{s}}{\partial r} \right)^{2} \left(\frac{1}{r \left(\frac{M_{s} \hat{V}_{s}}{V_{s}^{*}} + \hat{V}_{s}^{*} \right)} \right) \left(\frac{\phi_{s} M_{s} \hat{V}_{s}}{\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}^{*}\right)} \right) \left(\frac{\phi_{s} M_{s} \hat{V}_{s}}{\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \\ &+ \left(\frac{\partial \phi_{s}}{\left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \left(\phi_{s} \left(\hat{V}_{s} - \hat{V}_{s}\right) + M_{s} \hat{V}_{s}^{*}\right) - M_{s} \hat{V}_{s} \left(\hat{V}_{s} - \hat{V}_{s}\right) \right) \right) \\ &+ \left(\frac{\phi_{s}}{\phi_{s}} \left(M_{s} \hat{V}_{s} - M_{s} \hat{V}_{s}\right) + M_{s} \hat{V}_{s}^{*} - \frac{\phi_{s} \hat{V}_{s}}{\phi_{s} \left(\hat{V}_{s} - \hat{V}_{s}\right) + \hat{V}_{s}} \right) \right) \left(\frac{\phi_{s}}{\phi_{s}} \left(M_{s} \hat{V}_{s} - \tilde{V}_{s}\right) + \tilde{V}_{s}}{\left(\phi_{s} \left(\hat{V}_{s} - \tilde{V}_{s}\right) + \tilde{V}_{s} \left(\hat{V}_{s} \left(\hat{V}_{s} - \tilde{V}_{s}\right) + M_{s} \hat{V}_{s}} \right) \right) \right) \right) \\ \\ &+ \left(\frac{\phi_{s}}}{\phi_{s}} \left(M_{s} \hat{V}_{s}^{*} - M_{s} \hat{V}_{s}^{*}\right) + M_{s} \hat{V}_{s}^{*} - \frac{\phi_{s} \hat{V}_{s}}{\phi_{s} \left(\hat{V}_{s} - \hat{V}_{s}\right) + \tilde{V}_{s}} \right) \right) \left(\frac{1}{r \left(\frac{P_{am}}{V_{s}} \left(\hat{V}_{s} - \tilde{V}_{s}\right) + \tilde{V}_{s}} \left(\hat{V}_{s} - \tilde{V}_{s}\right) + (\ln r - \ln r_{m})}{\left(\phi_{s} \left(\hat{V}_{s} - \tilde{V}_{s}\right) + (\ln r - \ln r_{m})}\right) \right) \right) \right) \right) \right) \right) \\ \\ \\ &+ \left(\frac{\phi_{s}$$

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A.3.10 Volume flux revisited

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Here is the volume flux equation (A.4), with appropriate substitutions.

$$n_{a}^{*} = \left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)\left\{\frac{-\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)}\right\}$$

$$D_{ab}\left[\frac{\frac{\partial\phi_{a}}{\partial r}\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)}{\left(\phi_{a}\left(M_{b}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}+\left(\frac{\phi_{a}M_{b}\hat{V}_{b}}{\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}}-\frac{\phi_{a}\hat{V}_{b}}{\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}}\right)\left(\frac{1}{r\left(\frac{p_{atm}}{v_{\theta}^{*2}}\left(\hat{V}_{a}+\hat{V}_{b}\right)+\left(\ln r-\ln r_{\min}\right)\right)}\right)\right)\right\}\right)$$
(A.53)

Equation (A.53) may be rearranged and simplified.

$$n_{a}^{*} = -D_{ab} \begin{bmatrix} \frac{\partial \phi_{a}}{\partial r} \\ + \left(\frac{\phi_{a}M_{b}\hat{V}_{b}\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)} - \frac{\phi_{a}\hat{V}_{b}\left(\phi_{a}\left(M_{b}\hat{V}_{b}-M_{a}\hat{V}_{a}\right)+M_{a}\hat{V}_{a}\right)^{2}}{\left(M_{a}\hat{V}_{a}M_{b}\hat{V}_{b}\right)\left(\phi_{a}\left(\hat{V}_{b}-\hat{V}_{a}\right)+\hat{V}_{a}\right)} \right) \left(\frac{1}{r\left(\frac{p_{atm}}{v_{\theta}^{*2}}\left(\hat{V}_{a}+\hat{V}_{b}\right)+\left(\ln r-\ln r_{\min}\right)\right)} \right) \right) \right)$$
(A.54)

APPENDIX B

ADDITIONAL BOVINE-BLOOD GRAPHS

Here may be found the graphical results of bovine blood separation runs that were not included as examples within the body of the dissertation. These graphs, which we call accumulated cell-fraction graphs, represent the results of separation runs conducted on the NJIT Biotechnology Laboratory's V-II centrifuge.



Figure B-1 Bovine blood separation run B19890425a.

Separation run parameters:blood collected 5 day(s) prior to run;initial hematocrit, HCT=36.00;centrifuge spin speed, RPM=1380 min⁻¹;batch duration, θ =1380 s;separation quality, SQ=0.3274.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating theseparation: The X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.



Figure B-2 Bovine blood separation run B19890426a.

Separation run parameters:blood collected 6 day(s) prior to run;initial hematocrit, HCT=36.20;centrifuge spin speed, RPM=1350 min⁻¹;batch duration, θ =3300 s;separation quality, SQ=0.3607.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating theseparation: The X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.



Figure B-3 Bovine blood separation run B19890505a.

Separation run parameters:blood collected 1 day(s) prior to run;
centrifuge spin speed, RPM=1010 min⁻¹;
separation quality, SQ=0.3208.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating the
separation: The X=Y line corresponds to zero separation; the packed-RBC line represents ideal
RBC separation.



Figure B-4 Bovine blood separation run B19890508a.

Separation run parameters:blood collected 4 day(s) prior to run;initial hematocrit, HCT=42.20;centrifuge spin speed, RPM=1000 min⁻¹;batch duration, θ =3240 s;separation quality, SQ=0.2918.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating theseparation: The X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.



Figure B-5 Bovine blood separation run B19890510a.

Separation run parameters:blood collected 6 day(s) prior to run;
centrifuge spin speed, RPM=1040 min⁻¹;
separation quality, SQ=0.1428.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating the
separation: The X=Y line corresponds to zero separation; the packed-RBC line represents ideal
RBC separation.



Figure B-6 Bovine blood separation run B19890602a.

Separation run parameters:blood collected 1 day(s) prior to run;initial hematocrit, HCT=41.10;centrifuge spin speed, RPM=993 min⁻¹;batch duration, θ =3240 s;separation quality, SQ=0.3399.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating theseparation: The X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.



Figure B-7 Bovine blood separation run B19890605a.

Separation run parameters:blood collected 4 day(s) prior to run;initial hematocrit, HCT=31.50;centrifuge spin speed, RPM=1033 min⁻¹;batch duration, θ =1620 s;separation quality, SQ=0.4072.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating theseparation: The X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.



Figure B-8 Bovine blood separation run B19890620a.

Separation run parameters:blood collected 5 day(s) prior to run;initial hematocrit, HCT=39.50;centrifuge spin speed, RPM=800 min⁻¹;batch duration, θ =4500 s;separation quality, SQ=0.3367.The X=Y dashed line and packed-RBC dotted line provide visual guides for evaluating theseparation: The X=Y line corresponds to zero separation; the packed-RBC line represents idealRBC separation.
APPENDIX C

BOVINE SEPARATION RUN SUMMARIES

Tabular summaries of the bovine separation runs included in this investigation may be found in this appendix.

	Initial	Spin	Batch		WBC	RBC	
Run	HCT	Speed	Duration	Age	Balance	Balance	SQ
B19881212a	38.80	1161	1560	11	0.9131	0.9432	0.2069
B19890420a	37.00	1000	2820	0	1.5644	0.9839	0.3908
B19890421a	37.00	910	4740	1	1.0890	1.0212	0.4833
B19890424a	38.80	1019	2160	4	0.8402	0.9570	0.2920
B19890425a	36.00	1380	1380	5	0.9116	1.0816	0.3274
B19890426a	36.20	1350	3300	6	0.7755	1.0087	0.3607
B19890427a	6.43	900	2100	1	2.0280	4.4922	0.1869
B19890505a	37.70	1010	2940	1	1.5831	0.9519	0.3208
B19890508a	42.20	1000	3240	4	1.4685	1.0364	0.2918
B19890509a	38.80	810	4860	5	1.7555	1.1327	0.3057
B19890510a	11.30	1040	1380	6	0.7935	0.8065	0.1428
B19890602a	41.10	993	3240	1	0.9292	0.9270	0.3399
B19890605a	31.50	1033	1620	4	0.9922	1.0666	0.4072
B19890614a	8.02	860	1500	5	0.8550	1.0706	0.4253
B19890620a	39.50	800	4500	10	1.0303	0.9033	0.3367

 Table C-1 Summary of bovine separation runs—Chronological

•		<u> </u>	Detab			DBC	
	Initial	Spin	Batch		WBC	RDC	
Run	HCT	Speed	Duration	Age	Balance	Balance	SQ
B19890427a	6.43	900	2100	1	2.0280	4.4922	0.1869
B19890614a	8.02	860	1500	5	0.8550	1.0706	0.4253
B19890510a	11.30	1040	1380	6	0.7935	0.8065	0.1428
B19890605a	31.50	1033	1620	4	0.9922	1.0666	0.4072
B19890425a	36.00	1380	1380	5	0.9116	1.0816	0.3274
B19890426a	36.20	1350	3300	6	0.7755	1.0087	0.3607
B19890420a	37.00	1000	2820	0	1.5644	0.9839	0.3908
B19890421a	37.00	910	4740	1	1.0890	1.0212	0.4833
B19890505a	37.70	1010	2940	1	1.5831	0.9519	0.3208
B19881212a	38.80	1161	1560	11	0.9131	0.9432	0.2069
B19890424a	38.80	1019	2160	4	0.8402	0.9570	0.2920
B19890509a	38.80	810	4860	5	1.7555	1.1327	0.3057
B19890620a	39.50	800	4500	10	1.0303	0.9033	0.3367
B19890602a	41.10	993	3240	1	0.9292	0.9270	0.3399
B19890508a	42.20	1000	3240	4	1.4685	1.0364	0.2918

Table C-2 Summary of bovine separation runs—increasing initial hematocrit

	2	*					
	Initial	Spin	Batch		WBC	RBC	
Run	нст	Speed	Duration	Age	Balance	Balance	SQ
B19890620a	39.50	800	4500	10	1.0303	0.9033	0.3367
B19890509a	38.80	810	4860	5	1.7555	1.1327	0.3057
B19890614a	8.02	860	1500	5	0.8550	1.0706	0.4253
B19890427a	6.43	900	2100	1	2.0280	4.4922	0.1869
B19890421a	37.00	910	4740	1	1.0890	1.0212	0.4833
B19890602a	41.10	993	3240	1	0.9292	0.9270	0.3399
B19890508a	42.20	1000	3240	4	1.4685	1.0364	0.2918
B19890420a	37.00	1000	2820	0	1.5644	0.9839	0.3908
B19890505a	37.70	1010	2940	1	1.5831	0.9519	0.3208
B19890424a	38.80	1019	2160	4	0.8402	0.9570	0.2920
B19890605a	31.50	1033	1620	4	0.9922	1.0666	0.4072
B19890510a	11.30	1040	1380	6	0.7935	0.8065	0.1428
B19881212a	38.80	1161	1560	11	0.9131	0.9432	0.2069
B19890426a	36.20	1350	3300	6	0.7755	1.0087	0.3607
B19890425a	36.00	1380	1380	5	0.9116	1.0816	0.3274

Table C-3 Summary of bovine separation runs-increasing centrifuge spin speed

	Initial	Spin	Batch		WBC	RBC	
Run	нст	Speed	Duration	Age	Balance	Balance	SQ
B19890510a	11.30	1040	1380	6	0.7935	0.8065	0.1428
B19890425a	36.00	1380	1380	5	0.9116	1.0816	0.3274
B19890614a	8.02	860	1500	5	0.8550	1.0706	0.4253
B19881212a	38.80	1161	1560	11	0.9131	0.9432	0.2069
B19890605a	31.50	1033	1620	4	0.9922	1.0666	0.4072
B19890427a	6.43	900	2100	1	2.0280	4.4922	0.1869
B19890424a	38.80	1019	2160	4	0.8402	0.9570	0.2920
B19890420a	37.00	1000	2820	0	1.5644	0.9839	0.3908
B19890505a	37.70	1010	2940	1	1.5831	0.9519	0.3208
B19890508a	42.20	1000	3240	4	1.4685	1.0364	0.2918
B19890602a	41.10	993	3240	1	0.9292	0.9270	0.3399
B19890426a	36.20	1350	3300	6	0.7755	1.0087	0.3607
B19890620a	39.50	800	4500	10	1.0303	0.9033	0.3367
B19890421a	37.00	910	4740	1	1.0890	1.0212	0.4833
B19890509a	38.80	810	4860	5	1.7555	1.1327	0.3057

Table C-4 Summary of bovine separation runs-increasing batch duration

		- · · · · · · · · · · · · · · · · · · ·			0 1	1 2 4 2	
•	Initial	Spin	Batch		WBC	RBC	
Run	нст	Speed	Duration	Age	Balance	Balance	SQ
B19890510a	11.30	1040	1380	6	0.7935	0.8065	0.1428
B19890427a	6.43	900	2100	1	2.0280	4.4922	0.1869
B19881212a	38.80	1161	1560	11	0.9131	0.9432	0.2069
B19890508a	42.20	1000	3240	4	1.4685	1.0364	0.2918
B19890424a	38.80	1019	2160	4	0.8402	0.9570	0.2920
B19890509a	38.80	810	4860	5	1.7555	1.1327	0.3057
B19890505a	37.70	1010	2940	1	1.5831	0.9519	0.3208
B19890425a	36.00	1380	1380	5	0.9116	1.0816	0.3274
B19890620a	39.50	800	4500	10	1.0303	0.9033	0.3367
B19890602a	41.10	993	3240	1	0.9292	0.9270	0.3399
B19890426a	36.20	1350	3300	6	0.7755	1.0087	0.3607
B19890420a	37.00	1000	2820	0	1.5644	0.9839	0.3908
B19890605a	31.50	1033	1620	4	0.9922	1.0666	0.4072
B19890614a	8.02	860	1500	5	0.8550	1.0706	0.4253
B19890421a	37.00	910	4740	1	1.0890	1.0212	0.4833

Table C-5 Summary of bovine separation runs—increasing separation quality (SQ)

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CHAPTER D

BLOOD COUNTER SPECIFICATIONS

SysmexTMCC-180 Microcell Counter

The Sysmex[™] CC-180 is a semi-automated hematology analyzer for *in vitro* diagnostic

use in clinical laboratories. It provides the following eight analysis parameters.

- 1. White Blood Cell (WBC) or leukocyte Count
- 2. Red Blood Cell (RBC) or Erythrocyte Count
- 3. Hemoglobin (Hgb) Concentration
- 4. Hematocrit (Hct), true relative percentage volume of erythrocytes
- 5. Mean Corpuscular (Erythrocyte) Volume (MCV)
- 6. Mean Corpuscular (Erythrocyte) Hemoglobin (MCH)
- 7. Mean Corpuscular (Erythrocyte) Hemoglobin Concentration (MCHC)
- 8. Platelet (PLT) Count

Specifications

Name: Microcell Counter

Model Number: CC-180

Parameters: RBC, WBC, Hgb, Hct, MCV, MCH, MCHC, and PLT

Principles,

- WBC, RBC, & PLT: electric resistance detection
- Hemoglobin: Cyanmethemoglobin; light absorbance at 535 nm center frequency, with a glass bandpass filter, approximately 60 nm.
- Hematocrit: RBC signals converted to the pulse heights according to the sizes, and accumulated to give Hct value.
- MCV: computed from RBC and Hct
- MCH: computed from RBC and Hgb
- MCHC: computed from Hct and Hgb

Accuracy,

- RBC: error less than $\pm 2.5\%$
- WBC: error less that $\pm 3.0\%$
- Hematocrit: error less than ±2.0 Hct%
- PLT: error less than $\pm 5.0\%$
- (Except for Hematocrit, accuracy is determined by comparing the test results of a normal control blood obtained on a CC-180 with those obtained on a standard instrument. Hematocrit accuracy is determined by comparing the results with those obtained on the centrifuged microhematocrit method.)

Precision,

- RBC: reproducibility C.V. less than 1.0%
- WBC: reproducibility C.V. less than 1.5%
- Hemoglobin: reproducibility C.V. less than 1.0%
- Hematocrit: reproducibility C.V. less than 1.0%
- PLT: reproducibility C.V. less than 1.0%
- (C.V.: A coefficient of variation is calculated from five consecutive counts by recounting a normal control blood.)
- Linearity,
- RBC: Deviation $\pm 3\%$ for the range of $0.00 9.99 \times 10^6 / \text{mm}^3$
- WBC: Deviation $\pm 3\%$ for the range of $0.0 99.9 \times 10^3$ / mm³
- Hemoglobin: Deviation ±0.2g/dl for the range of 0.0-20.0g/dl, or Deviation ±0.3g/dl for the range of 20.1-30.0g/dl
- Hematocrit: Deviation $\pm 3\%$ for the range of 0.0 60.0%
- PLT: Deviation ±10×10³/mm³ for the range of 50-100×10³/mm³, or Deviation ±5% for the range of 101-600×10³/mm³
- (Condition: RBC count is in the range of $2.01 5.99 \times 10^6 / \text{mm}^3$.)
- (Conditions: (1) The background count is assumed as zero. (2) When counting in the range of RBC less than or equal to 0.50×10⁶/mm³, or Hgb less than or equal to 1.0g/d1, the sample I.D. number is set to zero so that the sample threshold limit will not function.)

Detection Sensitivity,

- WBC: Cell size over 4μ in diameter for 100μ aperture
- RBC: Cell size over 4μ in diameter for 80μ aperture
- Hemoglobin: 0.1g/dl
- Hematocrit: 0.1 Hct%
- PLT: Cell size over 1.6μ in diameter for 80μ aperture

Counting Time,

- Hemoglobin: approximately 8 seconds per test
- WBC: approximately 11.5 seconds per test
- RBC: approximately 19 seconds per test

Sample Volume,

- For counting cells: $0.50 \text{ml} \pm 1\%$
- For Hgb determination: approximately 3.2 ml
- Assuming a whole blood WBC count of 5000/mm³, a total of 5,000 cells are counted.
- Assuming a whole blood RBC count of 5,000,000/mm³, a total of 50,000 cells are counted.

APPENDIX E

HUMAN BLOOD PROPERTIES

	Male	Both	Female
WBC	$2.5 - 8.0 \times 10^3 / \mu L$		$2.5 - 9.0 \times 10^3 / \mu L$
WBC-SCR		$16.0 - 40.0 / \mu L$	
WBC-SCC		$0.6 - 2.4 / \mu L$	
WBC-MCR		$0.0 - 14.0 / \mu L$	
WBC-MCC		$0.0 - 0.8 / \mu L$	
WBC-LCR		$48 - 80 / \mu L$	
WBC-LCC		$1.1 - 5.7 / \mu L$	
RBC	$4.40 - 5.80 \times 10^6 / \mu L$		$3.90 - 5.30 \times 10^6 / \mu L$
HGB	$13.0 - 16.5 \mathrm{g/dL}$		11.5 - 14.7 g/dL
HCT	38-48%		34 - 44%
MCV	75 - 92 fL		76 – 92 fL
MCH	25 – 32 pg		24 – 31 pg
MCHC		$32.0 - 36.0 \mathrm{g/dL}$	
PLT		$150 - 400 \times 10^3 / \mu L$	
RDW SD	35 – 45 fL		35–47 fL
RDW CV		11.5-14.5%	
MPV		8.8-12.0 fL	

 Table E-1 Hemogram—Hematology Normal Values

Source (Tables E-1–E-4): Walters, Jerelyn G. and Patricia F. Garrity. 1987. *Case studies in the new morphology*. American Scientific Products: McGaw Park, IL p150.

Table E-2 Manual Differential

seg percent	14-62
band percent	0–10
lymph percent	25-40
mono percent	0-14
eos percent	0–6
baso percent	0–2
reticulocyte count	0.5-1.5%
solubility	negative

Table E-3 Hemostasis[†] normal values

prothrombin time	11.0–13.0 sec
activated partial thromboplastin time	23.0-34.0 sec
fibrinogen normal	150-400 mg/dL
fibrin degradation products	< 10 ug/mL
bleeding time	2.5–9.5 min

⁺ The stoppage of bleeding or hemorrhage: blood clotting.

Table	E-4	Chemistry	normal	values
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creatinine [†]	0.0-1.3 mg/dL
blood urea nitrogen	$10.0-20.0\mathrm{mg/dL}$
total bilirubin	
adult	$0.0 - 1.0 \mathrm{mg/dL}$
neonate	1.0-10.5 mg/dL
methemoglobin	1.0-10.5
alkaline phospatase	43.0–122.0 u/L
protein total	6.0 - 7.9 g/dL
albumin	3.4-4.6g/dL
serum vitamin B12	$>200 \mathrm{pg/mL}$
serum folate	>2.0 ng/mL
serum iron	35-145 ng/dL
serum UIBC	$100-325\mathrm{ng/dL}$
serum ferritin	$36-262\mathrm{ng/dL}$
AST	5.0-35.0 U/L
ALT	7.0–56.0 U/L
LD	297–537 U/L
GGT	8–52 U/L
FEP	$0-59 \mathrm{uL/dL}$
(free erythrocyte protoporphyrin)	,

^{\dagger} A creatine anhydride, C₄H₇N₃O, formed by the metabolism of creatine, that is found in muscle tissue and blood and normally excreted in the urine as a metabolic waste.

Table E-5 P	hvsical r	properties of human	blood	(normal	adult mean	values)
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Whole	blood
pH	7.35–7.40
Viscosity (37°C)	3.0 cP (at high shear rates)
Specific gravity (25/4°C)	1.056
Venous hematocrit: Male	0.47
Female	0.42
Whole blood volume	~78 mL/kg body wt
Plasma (o	pr serum)
Colloid osmotic pressure	~330 mm H ₂ O
рН	7.3–7.5
Viscosity (37°)	1.2 cP
Specific gravity (25/4°C)	1.0239
Formed e	elements
Erythre	ocytes
pH	7.396
Specific gravity (25/4°C)	1.098
Count: Male	5.4×10^9 /mL whole blood
Female	4.8×10^9 /mL whole blood
Mean corpuscular volume	$87 \mu \mathrm{m}^3$
Diameter	8.4 μm
Maximum thickness	2.4 μm
Minimum thickness	1.0 μm
Surface area	$163 \ \mu m^2$
Life span	120 days
Production rate	4.5×10^7 /mL whole blood
Hemoglobin concentration	0.335 g/mL whole blood per day
Leuko	cytes
Count	$\sim 7.4 \times 10^6$ /mL whole blood
Diameter	7–20 µm
Plate	elets
Count	$\sim 2.8 \times 10^8 / \text{mL}$ whole blood
Diameter	~2–5 µm

Source: Cooney, David O. 1976. Biomedical engineering principles: an introduction to fluid, heat, and mass transport processes. New York: Marcel Dekker. p39.

APPENDIX F '

VAMPIRE 3 CENTRIFUGE HEAD



APPENDIX G

SEPARATION QUALITY CORRELATION GRAPHS



Figure G-1 Separation Quality Correlation to Batch Duration (Bovine Blood Runs)

Bovine Blood Runs



Figure G-2 Separation Quality Correlation to Initial Hematocrit (Bovine Blood Runs)

Bovine Blood Runs



Figure G-3 Separation Quality Correlation to Spin Speed (Bovine Blood Runs)

Human Blood Runs



Figure G-4 Separation Quality Correlation to Batch Duration (Human Blood Runs)

GLOSSARY '

- Adoptive Transfer—Form of passive immunization where previously sensitized immunologic agents (cells or serum) are transferred to non-immune recipients. When transfer of cells is used as a therapy for the treatment of neoplasms, it is called adoptive immunotherapy (immunotherapy, adoptive).
- Anticoagulant— <haematology, pharmacology> Any substance that prevents blood clotting.

Those drugs administered for prophylaxis or treatment of thromboembolic disorders are heparin, which inactivates thrombin and several other clotting factors and which must be administered parenterally and the oral anticoagulants (warfarin, dicumarol and congeners) which inhibit the hepatic synthesis of vitamin K dependent clotting factors.

Anticoagulant solutions used for the preservation of stored whole blood and blood fractions are acid citrate dextrose (ACD), citrate phosphate dextrose (CPD), citrate phosphate dextrose adenine (cPDA 1) and heparin.

Anticoagulants used to prevent clotting of blood specimens for laboratory analysis are heparin and several substances that make calcium ions unavailable to the clotting process, including EDTA (ethylenediaminetetraacetic acid), citrate, oxalate and fluoride.

- Basophils— Granular leukocytes characterized by a relatively pale-staining, lobate nucleus and cytoplasm containing coarse dark-staining granules of variable size and stainable by basic dyes.
- Blood— <haematology> Considered a circulating tissue composed of a fluid portion (plasma) with suspended formed elements (red blood cells, white blood cells, platelets).
- Buffy coat <haematology>—Thin yellow white layer of leucocytes on top of the mass of red cells when whole blood is centrifuged.
- Buffy coat—the coagulated plasma of blood when the red corpuscles have so settled out that the coagulum appears nearly colorless. This is common in diseased conditions where the corpuscles run together more rapidly and in denser masses than usual. --Huxley.
- Complement activation—The sequential activation of serum components c1–c9, initiated by an erythrocyte-antibody complex or by microbial polysaccharides and properdin, and producing an inflammatory response.
- Cytapheresis— Separation of one or more kinds of cells from whole blood with the return of other blood cell constituents to the patient or donor. This is accomplished with an instrument that uses centrifugation to separate the cells into different layers based on the differences in cell density (displacement) or drag coefficients in a current (elutriation). The procedure is commonly used in adoptive transfer to isolate nk cells, lymphocytes, or monocytes.

- Elutriation— <ecology> Separation of particles on the basis of their differential sedimentation rate.
- Embolization— A treatment that clogs small blood vessels and blocks the flow of blood, such as to a tumor.
- Eosinophils— A type of polymorphonuclear leukocyte containing eosin-staining granules. Although the activity of eosinophils is not entirely clear, they are known to destroy parasitic organisms and play a major role in allergic reactions. They also secrete chemical mediators that can cause bronchoconstriction in asthma. Eosinophils make up one to three percent of the total white blood cell count.

Erythrocyte— A red blood cell.

- Granulocyte— <haematology> Leukocyte with conspicuous cytoplasmic granules. In humans the granulocytes are also classified as polymorphonuclear leucocytes and are subdivided according to the staining properties of the granules into eosinophils, basophils and neutrophils (using a Romanovsky type stain), some invertebrate blood cells are also referred to, not very helpfully, as granulocytes.
- Hematocrit— <haematology, investigation> Relative volume of blood occupied by erythrocytes. An average figure for humans is 45ml per cent, i.e. A packed red cell volume of 45ml in 100ml of blood.

(also haematocrit)—Relative volume of blood occupied by erythrocytes. An average figure for humans is 45 ml per cent, i.e. A packed red cell volume of 45ml in 100ml of blood.

Heparin— <drug> Sulphated mucopolysaccharide, found in granules of mast cells, that inhibits the action of thrombin on fibrinogen by potentiating antithrombins, thereby interfering with the blood clotting cascade. Platelet factor IV will neutralise heparin.

Leucoembolization—embolization caused by leukocytes (WBCs).

Leukapheresis— The preparation of leukocyte concentrates with the return of red cells and leukocyte-poor plasma to the donor.

Leukocyte—White blood cell.

Lymphocytes—White blood cells that fight infection and disease.

Lymphokine— <growth factor> Substance produced by a leukocyte that acts upon another cell.

Examples are interleukins, interferon alpha, lymphotoxin (tumour necrosis factor alpha), granulocyte monocyte colony stimulating factor (GM-CSF). The term is becoming less common and cytokine, a more general term, is taking over. Cytokines include lymphokines.

Lysing— lyse (ls, lz) v. intr. and tr. lysed, lysing, lysies. To undergo or cause to undergo lysis.

Lysis— <cell biology> Rupture of cell membranes and loss of cytoplasm.

- Macrophages, Round, granular, mononuclear phagocytes found in the alveoli of the lungs. They ingest small inhaled particles resulting in degradation and presentation of the antigen to immunocompetent cells.
- Macrophages—Relatively long lived phagocytic cell of mammalian tissues, derived from blood monocyte. Macrophages from different sites have distinctly different properties. Main types are peritoneal and alveolar macrophages, tissue macrophages (histiocytes), Kupffer cells of the liver and osteoclasts. In response to foreign materials may become stimulated or activated. Macrophages play an important role in killing of some bacteria, protozoa and tumour cells, release substances that stimulate other cells of the immune system and are involved in antigen presentation. May further differentiate within chronic inflammatory lesions to epithelioid cells or may fuse to form foreign body giant cells or Langhans giant cells.
- Mitochondria—<cell biology> A small intracellular organelle which is responsible for energy production and cellular respiration.
- Monocyte—<haematology> Mononuclear phagocyte circulating in blood that will later emigrate into tissue and differentiate into a macrophage.
- Monocytes—One of three types of white blood cells. Monocytes are precursors to macrophages.
- Mononuclear Phagocyte—<haematology> Monocytes and their differentiated products, macrophages. Mononuclear cells are leucocytes other than polymorphonuclear cells and include lymphocytes.
- Neutropenia— <haematology> Leucopenia in which the decrease in white blood cells is chiefly in neutrophils.
- Neutrophils— Granular leukocytes having a nucleus with three to five lobes connected by slender threads of chromatin, and cytoplasm containing fine inconspicuous granules and stainable by neutral dyes.
- Plasmapheresis— <procedure> Centrifuging blood that has been removed from the body to separate the cellular elements from the plasma.
- Platelet—<haematology> A discoid cell (3μ m diameter) found in large numbers in blood, important for blood coagulation and for haemostasis by repairing breaches (small breaks) in the walls of blood vessels.

Platelet granules contain lysosomal enzymes, dense granules contain ADP (a potent platelet aggregating factor) and serotonin (a vasoactive amine). They also release platelet-derived growth factor which presumably contributes to later repair processes by stimulating fibroblast proliferation.

Synonym: thrombocytes.

Polymorphonuclear Leucocytes — Mammalian blood leucocyte (granulocyte) of myeloid series in distinction to mononuclear leucocytes: See: neutrophil, eosinophil, basophil.

- Red blood cell— <haematology> Cell specialized for oxygen transport, having a high concentration of haemoglobin in the cytoplasm (and little else). Biconcave, anucleate discs, 7μ m diameter in mammals, nucleus contracted and chromatin condensed in other vertebrates. Acronym: RBC
- Romanovsky type stain—<technique> Composite histological stains including methylene blue, Azure A or B and eosin, sometimes with other stains.
- Rouleau— <haematology> Cylindrical masses of red blood cells. Horse blood will spontaneously form rouleaux, in other species it can be induced by reducing the repulsion forces between erythrocytes.
- sedimentation rate, or sed rate, is a blood test that detects and monitors inflammation activity. It is measured by recording the rate at which red blood cells (rbcs) sediment in a tube over time. It increases (the rbcs sediment faster) with more inflammation.
- White blood cells (WBCs) are cells which circulate in the blood and lymphatic system and harbor in the lymph glands and spleen. They are part of the immune system responsible for both directly (t cells and macrophages) and indirectly (b cells producing antibodies) attacking foreign invaders of the body.
- White Blood Cell <haematology> White corpuscles in the blood. They are spherical, colorless and nucleated masses involved with host defenses.

Normal white blood cell counts are variable with age and sex. Normal adult range is 4, 500 to 11,000 cells per cubic millimetre of blood. Slightly higher counts are seen in children. Elevated counts can be seen in cases of inflammation and infection.

Acronym: WBC

On-line Medical Dictionary

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