Characterization of Shear-Induced Hemolysis in Rotational Medical Devices

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Characterization of Shear-Induced Hemolysis in Rotational Medical Devices

By

James A. Krisher

A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Mechanical Engineering

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Abstract

The implantation of a Left Ventricular Assist Device (LVAD) is a rapidly growing means of treatment for a large variety of heart ailments. These blood pumps necessarily exert some degree of shear stress on blood passing through them, which, over time, may cause the rupture of red blood cells, coagulation, thromboses, and bleeding. This limits their use as long term treatment and therapy. While the relationship of fluid shear to blood damage has been demonstrated previously, there is significant variance in the reported levels of damage. Were these stresses and subsequent damage to be well understood and related, existing and future VADs, as well as other blood contacting devices, could be more quantitatively designed to apply shear stresses below some damage threshold, thus allowing for longer effective treatment for an implanted patient.

The aim of this thesis has been to construct a robust, Couette-flow shearing device in order to expose blood to a known shear stress under well controlled experimental conditions and to evaluate the subsequent damage. This shearing device has been constructed from an existing axial flow LVAD design that uses a novel magnetic bearing system to levitate the pump’s rotor. This technology gives the shearing device a uniquely simplified flow path and avoids any stresses contributed by conventional bearings. Both ovine and porcine blood has been investigated in the course of this work over a stress range of 0-350Pa and exposure time ranging from 200-1200ms. Extremely low levels of damage relative to studies in the literature were observed below 200Pa for both species over any exposure time. Above those conditions, porcine blood shows signs of higher fragility than ovine, seeing as much as 1% red blood cell damage within the investigated range, while ovine damage under identical conditions remained below 0.2%.
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Nomenclature

Abbreviations

LVAD  Left Ventricular Assist Device
VAD   Ventricular Assist Device
RBC   Red Blood Cell
Hb    Hemoglobin
fHb   Free Hemoglobin
pfHb  Plasma Free Hemoglobin
IH    Index of Hemolysis
Hct   Hematocrit
vWf   Von Willebrand Factor
CFD   Computational Fluid Dynamics
HESA  Hall Effect Sensor Array
AMB   Active Magnetic Bearing
PID   Proportional-Integral-Differential
FX    Front X-axis
FY    Front Y-axis
RX    Rear X-axis
RY    Rear Y-axis
PBS   Phosphate Buffered Saline
PIV   Particle Image Velocimetry
RIT   Rochester Institute of Technology
US FDA United States Food and Drug Administration
ASTM  American Society for Testing and Materials
L/R/C Left, Right, or Center position of a figure
Notation

\( \tau \) \quad \text{Shear Stress}

\( \dot{\gamma} \) \quad \text{Shear Rate}

\( t_{\text{exp}} \) \quad \text{Exposure Time}

\( U \) \quad \text{Constant Velocity}

\( u \) \quad \text{Non-constant Velocity}

\( a \) \quad \text{Gap Thickness}

\( \mu \) \quad \text{Dynamic Viscosity}

\( \nu \) \quad \text{Kinematic Viscosity}

\( R \) \quad \text{Constant Radius}

\( \omega / \Omega \) \quad \text{Angular Velocity}

\( V \) \quad \text{Volume}

\( L \) \quad \text{Length}

\( Q \) \quad \text{Volumetric Flowrate}

\( A_{\text{CS}} \) \quad \text{Cross-Sectional Area}

\( \eta \) \quad \text{Radius Ratio}

\( C/\alpha/\beta \) \quad \text{Experimentally Derived Constants}

\( \rho \) \quad \text{Density}

\( \Delta \) \quad \text{Uncertainty}

\( f \) \quad \text{Frequency}

\( \varepsilon \) \quad \text{Strain}

\( A \) \quad \text{Absorbance}

\( \text{Re} \) \quad \text{Reynolds Number}

\( \text{Ta} \) \quad \text{Taylor Number}
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1 Introduction

1.1 LVADs and Blood Damage

Left Ventricular Assist Devices (LVADs) are implanted blood pumps used in the treatment of patients with some degree of heart failure. Typically, this treatment is used as a bridge-to-transplant, meaning that the device is implanted for a short time while the patient waits for a donor heart to become available. LVADs are also growing in popularity as a destination therapy treatment, meaning that, in lieu of a transplant, the implanted pump is a permanent solution.

Figure 1 – Diagram of an implanted Heatmate II, Thorarec’s axial flow LVAD. [1] The device is grafted to the left ventricle and aorta and operates parallel to the heart’s natural function.

In blood-contacting medical devices, and in LVADs especially, fluid shear is generated by the blood’s movement through the device. A particle suspended in the blood, such as a red blood cell or platelet, experiences this as a mechanical stress and associated strain. This damages the particle and in high enough magnitude, leads to the rupture of...
cells. Stress acting on blood components is also associated with both clotting and bleeding, which is detrimental to the health of an implanted patient [2]–[7].

Shear-induced blood damage, and its associated symptoms, create complications in destination therapy LVAD patients and that increase rates of cardiac adverse events in even short-term bridge-to-transplant patients. Presently, there is a lack of consistent data characterizing the nature of the relationship in LVADs between the magnitude of applied shear, exposure time to that shear, and resulting damage. It is challenging to quantitatively describe the stresses applied in a modern VAD due to the complicated and dynamic flow paths found within a rotating pump as it pushes fluid. An alternative approach taken by some studies is to create a shearing device with simplified, well understood fluid paths within which blood can be exposed to a known shear stress [5], [6].

The goal with most innovations in modern pumps is simply to reduce stresses because a threshold value-of-safety has not been well agreed upon, though most investigators agree such a threshold exists. However, levels of shear that are non-physiologic and have the potential cause damage are, to date, unavoidable in prosthetic pumps. This is particularly true in the modern rotary type devices that have become much more popular than diaphragm devices because of their reliability. By studying and quantifying how much stress causes what degree of damage, modern pumps could be better designed by considering clear models for blood damage.

1.2 Flow Regimes and Mechanisms of Shear in Medical Devices

Couette flow is a model used to describe steady, viscous, laminar flow between two flat plates where one plate is moving relative to the other. The no-slip condition imposed
on the fluid at the surface of each plate means that the movement of one plate creates a linear velocity gradient, the slope of which is the shear rate imparted onto the fluid.

\[ \tau = \frac{du}{dx} \mu = \dot{\gamma} \mu = \frac{U \mu}{a} \]  \hspace{1cm} (1-1)

The same theory, when applied to a cylindrical model, is known as Taylor-Couette flow. Figure 3 depicts one instance of Taylor-Couette flow in which the inner of two concentric cylinders is rotating and the outer cylinder is stationary. As before, assuming laminar flow in the circumferential direction, the shear stress in the fluid can be expressed as a function of the rotational velocity, the fluid viscosity, and the gap size.

\[ \tau = \frac{du}{dr} \mu = \dot{\gamma} \mu = \frac{u(R_1) \ast \mu}{R_2 - R_1} = \frac{\omega R_1 \mu}{a} \]  \hspace{1cm} (1-2)
In instances of Taylor-Couette flow where the difference between inner and outer cylinder velocities is significantly positive, as in the proposed case, the radial centrifugal force causes the generation of wavy toroidal vortices in the axial direction of the annulus [8], [9]. These Taylor vortices have been a highly studied phenomenon in fluid mechanics, ever since their first characterization by G. I. Taylor in 1923.

![Diagram of Taylor vortices in an annulus with rotating inner cylinder][10]. Often the vortices are described using the size, number and spacing of the stratified flow.

In order to better quantify these vortices, a non-dimensional Taylor number is used with the suggestion that the vortices develop above some critical value. This is typically characterized in terms of the radius ratio, \( \eta = \frac{R_1}{R_2} \). The Taylor number for Taylor-Couette flow is given below.

\[
Ta = \frac{\Omega^2 R_1 (R_2 - R_1)^3}{v^2}
\]  

(1-3)

Since 1923, further complexities of Taylor-Couette flow have been studied and quantified. The so-called “rich flow structures” are sometimes represented in terms of the relative Reynolds numbers given by the velocities of the inner and outer cylinders [9], [11].

\[
Re_i = \frac{\Omega R_1 (R_2 - R_1)}{v} \quad Re_o = \frac{\Omega R_2 (R_2 - R_1)}{v}
\]  

(1-4)
In cases like the one proposed in Figure 3, the outer Reynolds number, $Re_o$, would be zero as the wall is static.

Figure 5 – Phase diagram of rich flow structures for Taylor-Couette flow with a radius ratio of $\eta = 0.833$ [9]. The dashed line at $Re_o = 0$ is the primary area of significance to this thesis.

Poisseuille flow, more colloquially know as pipe flow, is another regime describing pressure-driven fluid flow down a long duct or pipe. Unlike Couette flow, the velocity profile in Poisseuille flow is parabolic, meaning that it is at its maximum at the center of the channel and effectively zero at both walls due to the no-slip condition.

Figure 6 – Diagram of Poisseuille flow regime.
As in Couette flow, the derivative of the velocity profile is shear rate, which taken as a product with viscosity is the fluid shear stress. This means that the shear stress at the center of the channel is zero and that it is maximized where the velocity gradient is the steepest at the walls.

The previously described modes of shear stress, present in Taylor-Couette and Poiseuille flow, are categorized as viscous stresses. This is opposed to Reynolds stresses, which occur in turbulent flow as a result of small convections and eddies. The degree of turbulence induced blood damage is often studied using jet flow through a small nozzle or orifice and described in relation to the size and magnitude of the turbulent eddies. It has been suggested that distinction between these modes is important when considering the body of literature examining shear-induced blood damage in medical devices [12]. While both viscous and Reynolds stress are present in modern LVADs, this thesis will primarily focus on the analysis of the former as the Couette-shear fluid regimes under examination are laminar in the region of greatest significance and would not typically present levels of turbulence large enough to cause significant blood damage.

1.3 Red Blood Cells and Hemolysis

Erythrocytes, more commonly called red blood cells (RBC), are the largest solid portion of blood. Their primary function is the storage and transport of hemoglobin, the protein responsible for binding to oxygen and releasing it to cells throughout the body. The presence of this protein gives blood its red coloring. A red blood cell’s life cycle can be as long as 120 days in humans. Hematocrit (Hct) is a term used to describe the percentage of red cells in the plasma. In humans this is typically around 40% [13].
The shape of a red blood cell is a biconcave disk. This shape has very high deformability that allows the cell to squeeze into the small capillary vessels of the body. Humans RBCs are ~8µm in diameter and ~2µm thick. The shape is the same in other species, but size can change between species [14]. Under an applied shear, there are several modes of motion a cell can experience. These can be broadly categorized as tank-treading, tumbling, and deformational [15]. Tank-treading refers to the slipping of the cell membrane around the interior contents under applied shear, an action similar to the motion of a tank tread. Tumbling describes the motion of cells in a larger channel, wherein they flip end-over-end freely. Finally, under higher shear conditions, such as the naturally occurring Poisseuille shear in capillaries, a cell can undergo deformation. This is often described as either slipper or parachute deformation [15]–[19].


In instances where the applied shear is extreme relative to a cells normal biological experience, the cell can lyse, or rupture, causing cell contents to be released into the plasma. For red blood cells specifically, this process is called hemolysis. Hemolysis can be induced mechanically, by applied stress, or chemically, by the introduction of some compound that destroys the membrane. Or it can be induced by an osmotic pressure
gradient caused by suspension of the cell in a solution of unequal salinity. Certain conditions, such as high shear, can also cause pores in the cell membrane to grow and hemoglobin to leak out [3], [20]. In the regular biological function of a living animal, the fragments of red cells and the hemoglobin in the plasma are filtered and cleaned out by the spleen and liver [13].

![Diagram of hemolysis showing hemoglobin being released](image)

*Figure 8 – Diagram of hemolysis showing hemoglobin being released [21].*

The primary marker used for quantifying hemolysis in research applications is the plasma free hemoglobin (pfHb or simply fHb). A whole blood sample can be centrifuged to separate the cell fragments and other blood components from the liquid plasma, in which the free hemoglobin remains suspended. In the plasma, the hemoglobin manifests as a red tint, the intensity of which is proportional to the amount of damaged red blood cells from the original whole blood sample. In order to quantitatively measure the redness of the plasma, a spectrophotometer can be used to measure the absorbance at select wavelengths of light shined through the sample. This optical technique has been extensively practiced in blood damage literature and is an standard accepted by the US FDA and ASTM International [22]–[24].
However, it is also important to consider that for a given set of damaging conditions, the amount of plasma free hemoglobin observed in a sample is dependent upon the initial amount of cells, the hematocrit, and the initial total blood hemoglobin concentration (Total Hb). A commonly used method for compensating for these values between samples and between studies is to use the index of hemolysis (IH), which represents the level of hemolysis as a percentage of damage [3], [6], [26]–[28].

\[
\%IH = \frac{fHb[V(1 - Hct)]}{V(Total\ Hb)} \times 100 = \frac{fHb[(1 - Hct)]}{(Total\ Hb)} \times 100 \tag{1-5}
\]

In the event that the total blood hemoglobin concentration cannot be directly measured, one can approximate the concentration in g/dl as one third of the hematocrit [29].

\[
Total\ Hb \left[ \frac{g}{dl} \right] \approx \frac{Hct[\%]}{3} \tag{1-6}
\]

Red blood cell damage is not usually cited directly as the most clinically lethal risk in modern LVADs because clotting, bleeding, and infection all have much higher rates of incidence in implanted patients [30]–[32]. However, hemolysis is potentially a contributing factor to these events and is directly correlated with kidney damage. It is also recognized that red blood cells are among the least fragile of the blood components [3]. That being
said, there are primarily three reasons that justify the continued study of hemolysis in medical devices. The first reason is that hemolysis is a well validated and easily quantified metric of blood damage with an appropriate range and sensitivity to adequately indicate damage. Second, there is an overwhelmingly large body of literature studying and discussing methods and mechanisms of hemolysis. It is a mostly standardized metric of blood damage between investigative studies and commonly used as a design criteria for new devices as well as in their evaluation for regulatory purposes. Finally, red blood cell damage provides an upper-bound analysis of damage to other components. That is to say, if there is a significant degree of hemolysis, one can be certain there is also significant damage to other blood factors.

1.4 Platelets and Thrombosis

Thrombosis, commonly known as blood clotting, is a major complication that can occur as a result of implanted devices. A critical part of the clotting process is the activation of platelets - cell fragments with the natural function of clotting blood in order to repair damaged blood vessels and prevent bleeding. It has been shown that regions of non-physiological high shear stress can cause platelets to unnecessarily activate and initiate the clotting process on implanted surfaces, such as those in an LVAD. These activated platelets are then rendered less useful where and when they are needed, which can result in bleeding complications in patients. Similarly, the Von Willebrand Factor (vWF) is a protein component of whole blood and another critical actor in the clotting process. This too has been demonstrated susceptible to damage from shear stress in implanted blood-contacting medical devices. As stated above, the shear stress and exposure time threshold for
significant platelet activation, as well as for Von Willebrand Factor damage, has been demonstrated to be considerably lower than the threshold for hemolysis [3]. There have been several studies demonstrating the effectiveness of platelet activation state assays as used in shear damage experiments [4], [33]–[36].

1.5 **Blood Viscoelasticity**

Blood has an especially interesting non-Newtonian viscoelastic behavior. It is shear-thinning, meaning that as applied fluid shear rate increases, the bulk viscosity of the blood decreases. This is primarily a result of its nature as a suspension of easily deformable particles – red cells. Under increasingly high shear rates, the cells stretch out, as shown in Figure 7, which allows the plasma suspending them to flow more readily, decreasing the measured viscosity [37]–[40]. At very low shear rates, below 200s\(^{-1}\), there is a phenomenon known as erythrocyte aggregation, in which the red blood cells clump together in short stacks, called rouleaux [38]. These float around the blood behaving as much larger particles than a single cell and increase the apparent viscosity. As this low shear rate increases, the stacks separate and become smaller particles, reducing the apparent viscosity.

*Figure 10 – Microscope image of rouleaux [41]. The blood in this case is likely static or at very low shear flow.*
The shear-thinning quality of blood – both from aggregation and cell deformation – is most dramatic below shear rates around 1000s\(^{-1}\). The shear experienced in some locations within a modern VAD is orders of magnitude higher, so it is unlikely that the non-Newtonian characteristics affect the bulk flow properties. In fact, most design and modeling of pumps is based on a Newtonian model for the fluid.

Nonetheless, these are important considerations for any studies examining blood damage, particularly where the experimental evaluation of blood viscosity is necessary for use in stress calculation. In many devices used for blood damage study, the shear rate is known, but the fluid stress may only be calculated based on viscosity, which may vary throughout different regions of the system. Red cell dynamics cause whole blood to have elastic properties as well [42], [43]. The flexible cell membrane deforms and stores energy like a spring and can dampen the motion of the fluid. In a high speed rotating device, the forces generated by the fluid and acting onto the pump impeller have an effect on impeller dynamics. This is particularly important in a magnetic bearing LVAD where the impeller levitates in the blood and its position is continually controlled and affected by the viscoelastic fluid forces.

Because of the particulate nature of the red cells, there is another interesting phenomenon that occurs where the flow channel is very small. The Fahraeus effect describes how the high shear at the walls of a small blood vessel, given by the Poisseuille flow regime, causes the particulate cells to be pushed to the center of the channel via the Magnus effect. This creates a cell-free layer near the walls which is at a much lower viscosity than the densely clustered cells in the low shear region of the channel center. The
system could then be described as a viscously thick column of cells gliding on the much thinner plasma.

![Diagram of Fahraeus effect](image)

*Figure 11 – Diagram of Fahraeus effect [44]. Note the cell free layer on either side of the channel.*

As an extension of this, the Fahraeus-Lindqvist effect asserts that as diameter decreases, the apparent bulk viscosity also decreases in blood vessels with diameters less than 300µm. This property of blood has been extensively studied as it relates to blood flow in capillaries and other blood vessels of small diameter [37], [45], [46]. That said, there is still some dispute as to the impact of this effect in modern medical devices and on blood damage investigations relating to them [3], [6]. The shear is constant across an entire channel in a Couette flow regime so it is unlikely such a flow would cause the Fahraeus-Lindqvist effect to manifest. However, there is often a superimposed axial Poisseuille involved in typically studied flow geometries and that could potentially cause the Fahraeus-Lindqvist effect to occur and affect the bulk viscosity and observed flow structures, provided the channel is sufficiently small (<300µm).

Another extremely important consideration for studies in this field is blood temperature. Like most fluids, as temperature increases, viscosity decreases and in many devices with active electrical components, there is the potential for non-negligible heat generation which affects blood viscosity, both in *in-vitro* and *in-vivo* applications. Not only would a different temperature change the apparent viscosity at a given instant, it would change the degree of impact of the previously described phenomena. Colder red cells will...
likely deform to a different extent than warm cells would, causing a different shear-thinning character. Similarly, the bulk hematocrit is an important consideration for robust description of blood viscosity. As with temperature, different hematocrits will yield different instantaneous viscosities with all other parameters equal. And, like temperature, different hematocrits will also amplify or reduce the magnitude of other viscoelastic phenomena. For example, the Fahraeus-Lindqvist effect will be more significant in pools with very high hematocrit vs those with very low. Blood viscoelasticity is prone to significant variability from a myriad of factors, which makes it critical that any investigators appropriately characterize the blood viscosity in their experiment.

1.6 Existing Studies and Devices

A frequently applied mathematical model of hemolysis is the power-law model, published by Giersiepen et al. (1990) [2]. The model delivers the index of hemolysis as a function of shear stress and exposure time and takes the form shown in Equation (1-7).

\[
IH = \frac{\Delta f \cdot Hb}{HB} = \frac{C_{\tau_{\text{shear}}} \cdot t_{\text{exp}}^\alpha}{\tau_{\text{shear}}^\beta}
\]  

(1-7)

In this analysis, C, \( \alpha \), and \( \beta \) are constants derived from a fit to experimental data. This model assumes a laminar Couette flow in which the shear stress is constant along a given streamline, and thus the mechanical stress felt by a given red blood cell is constant for the duration of its exposure time [47]. While this is almost certainly not explicitly the case in a modern VAD, the Giersiepen power-law model is a useful tool for characterizing the key factors of hemolysis and quantifying and comparing their observed effect between experimental studies. It also allows effective computational predication of blood damage, provided there is an accurate computational flow model of stresses available and the
power-law constants in use are well validated and agreed upon by experimental investigation. There are several works investigating, via computational fluid dynamics (CFD), the validity of the power-law model and others, in accurately predicting hemolysis in simple geometries and in LVAD pumps [47], [48]. In all of the suggested models and associated adjustments, a recurring problem is the disparity between experimental studies on how much stress causes what degree of damage over a range of exposure times. The constants $C$, $\alpha$, and $\beta$ are not well agreed upon.

There have been several experimental investigations of stress-induced blood damage in devices that emulate modern VADs, primarily in that they use a Taylor-Couette flow for application of controlled shear stress. Zhang et al. (2012) adapted two LVAD devices, the axial flow Jarvik 2000 and the centrifugal flow CentriMag, in order to create simplified flow geometries that allowed for analytical analysis of the applied Couette flow stresses while still remaining characteristically representative of the geometries of the original pumps. The Jarvik 2000-analogue was used to examine stresses ranging from 120-340Pa while the CentriMag-analogue was used to examine stresses ranging from 20-200Pa, both over exposure times from 40ms to 1500ms.
Figure 12 – Diagram of devices used in Zhang (2012) and Ding (2015). (a) Jarvik 2000; (b) Jarvik-analogue shearing device; (c) CentriMag; (d) CentriMag-analogue shearing device [26].

Heparinized ovine blood was collected from a slaughterhouse via sacrificial cut and hemolysis was evaluated over the specified ranges. This led to a set of power-law coefficients suggesting lower blood fragility than those observed in Giersiepen’s hemolysis experiments [2], [26]. The Zhang (2012) power-law curve can be seen in Figure 13. This work was then continued using the same devices by Ding et al. (2015), where porcine, bovine, and human were examined for comparison to the ovine from Zhang (2012). Their results indicated that of the three animal species, ovine was the most fragile and porcine the least [27]. This disagrees with the findings of an ongoing study at the US FDA which observed that bovine and ovine red cells are considerably less fragile than larger porcine and human cells, though in that case the cells were damaged via a high pressure Poisseuille jet through a 150µm orifice, which may affect the direct comparability of their results [49].
Interestingly, the gradual shape of the curves in Zhang (2012) and Ding (2015) stands in contrast to one observed in a similar experiment years prior by Paul et al. (2003). In that experiment, venipuncture porcine blood was examined over a comparable range using a different style of Couette shearing device featuring a novel sealing fluid. Paul (2003) observed minimal damage across the entire observed range until a threshold of 425Pa and 600ms at which point hemolysis dramatically increased. The hemolysis results and a model of the device can be viewed in Figure 14.

Figure 13 – Power-law fits of Zhang/Ding hemolysis results. Ovine was conducted in Zhang (2012), all others in Ding (2015) [27]. Note that ovine is described to be the most fragile and porcine the least.
Boehning et al. (2014), a separate group, performed an experiment with an extremely similar device under comparable conditions with exception that they examined only two exposure times over a much higher range of stresses than in Paul (2003). Slaughterhouse heparinized porcine blood was examined and observed to have minimal damage at 50ms and an exponential curve of damage at 875ms, with 1% damage occurring beyond 600Pa [28]. Their device and results are shown in Figure 15.
It is likely that in each of these experiments as well as in many others in the literature, there have been secondary device characteristics and experiment variations that contribute to the differences in observed results. While it is difficult to precisely identify the factors at play, the device used, the species investigated, and the method of collection are likely culprits.

1.7 The RIT Lev-VAD

The Lev-VAD was developed at RIT as an axial flow LVAD with novel magnetic bearings, meaning that rather than using conventional bearings (often blood lubricated) as many other VAD’s do, the Lev-VAD’s rotor levitates in the housing tube. This simplifies the flow path and eliminates any friction and shear stress caused by conventional bearings that could influence blood damage. The Active Magnetic Bearing (AMB) system in the Lev-VAD stabilizes the rotor in the radial direction and ensures it is securely levitating on the central axis of the pump. The AMB’s are made up of four electromagnets which are energized to apply the necessary force to hold the rotor steady. These are Front-X, Front-Y, Rear-X, and...
Rear-Y (FX,FY,RX,RY). In the axial direction, passive magnetic forces hold the rotor in position. The radial position of the rotor is measured by two Hall-Effect Sensor Array (HESA) rings which are positioned similarly to the AMBs.

![Figure 16 – Section view of RIT Lev-VAD [50]. Note the axial position of the HESAs, AMBs, and motor.](image)

The radial rotor positions measured by the HESA’s are relayed into a Proportional-Integral-Differential (PID) controller which determines the required power to energize each AMB with in order to hold the impeller levitating on the axis. A brushless three-phase motor interacts with the impeller to apply torque to the impeller and provide the mechanical energy necessary to propel blood.

The lack of conventional bearings makes this device highly suitable for adaptation into a Taylor-Couette shear device like those used in the literature, most similarly the axial-flow shearing device used in Zhang (2012) and Ding (2015). Conventional bearings like those used in the literature complicate the flow path and add friction and heat to the system which could be a secondary source of unquantified shear and hemolysis in experiments which use them. A magnetic bearing device avoids these potential risks.
Chapter 1: Introduction

Figure 17 - Cross-section model of RIT magnetic bearing Couette shear device. Blood flows through the shearing gap annulus and is sheared at a rate given by the rotor speed.

Rather than attempt to construct a mag-lev shearing device from scratch, an operational Lev-VAD pump was partially deconstructed and modified to the geometry shown in Figure 17. The blades were removed from the Lev-VAD impeller and a tapered ring was fitted to it in order to decrease the annular gap width and allow the application of high-magnitude Couette shear as a function of the rotor speed. A syringe pump allows the controlled flow through the device in a single pass, with the flowrate being proportional to the blood’s exposure time to the region of high shear. This results in a finely tunable device which allows the characterization of hemolysis as a function of shear stress and exposure time in a device which is highly similar to modern axial flow LVAD’s.

There have been a number of other theses concerned with the development and application of this magnetic bearing shearing device. The immediate predecessor to this current work was a thesis completed by Raghunathan (2014). In that work, the technology of the RIT Lev-VAD was used to construct a magnetic bearing Couette shearing device from available components. Unfortunately, the device in that work was not robust enough for rigorous blood testing. Most significantly, the hemolysis experiment was inhibited by extreme overheating in the electromagnetic coils of the motor and magnetic bearings due to insufficient cooling. There was also a good deal of exposed electronics which were not
waterproofed and had a high rate of breakage from routine handling. Several critical experiment parameters were left uncharacterized which discredits the small amount of hemolysis data that was able to be collected [51]. Still, much of the conceptual and design effort of Raghunathan (2014) informed the construction of the new iteration of the mag-lev shearing device and experiment apparatus constructed in this thesis.
2 Motivation

2.1 Disparity in Literature

Despite the numerous studies attempting to characterize blood fragility, there is very little agreement on a firm stress threshold value that could be used by device designers to avoid or reduce stress, and by extension damage, to a level that is manageable by the human body. From a broad perspective over all of the stress induced blood damage literature as it relates to medical devices, there is little to no agreement on the predicted hemolysis for any given combination of fluid shear and exposure time. Moreover, the range of necessary investigation lacks boundaries. It is likely that a cell can endure an extremely high shear provided the exposure is sufficiently short and vice versa, however the field is lacking definition of where these boundaries lie. Still other questions in this area remain disparate. For example, when testing with animal blood, which species is most fragile and how much more so than other species? Which best simulates the red cell fragility of human blood? A handful of studies have investigated these questions and have arrived at differing answers.

It is likely that the documented disparity is primarily a result of experiment variation between research groups. Some studies employ Couette flow shearing devices modeled after an axial flow LVAD where others use devices resembling a centrifugal LVAD, each with their own novelties and quirks. There are also investigations of blood damage using Poisseuille flow shearing devices pushing blood through a fine nozzle at high
Chapter 2: Motivation

pressure and microfluidic devices examining cell deformation and damage as they pass through a microscale channel. There are different methodologies used to quantify the applied stress and different assumptions made about the significance of viscosity, temperature, blood species etc. Each study, regardless of the apparatus and assays used, has made a valid contribution to the understanding of stress induced blood damage. While no single study can unequivocally answer the posed questions, each can offer useful observations about the behavior and fragility of blood and red cells within the context of that experiment.

This thesis does not attempt to reconcile or explain the differences between studies in their entirety (though considerations to this effect will be discussed in chapter 1). Nor will it report an exact, one-to-one replication of any one of these experiments. Rather, the aim has been to perform a similar experiment with similar analysis to those found in the literature with an emphasis on a robust, well characterized, and well documented test apparatus and methodology in a flow field that has direct relevance to the flow within a rotational VAD. The hope is not that this work will totally resolve any of the open blood damage questions, but that it will provide a useful point of comparison to existing and future studies in the field and shed light on reasons for agreement and incongruences between works.

2.2 The Merits of Couette-Shear Devices

First and foremost, Couette flow is the primary mechanism of shear stress in modern VADs and as such it is useful to duplicate this flow regime when studying blood damage as it relates to LVADs. Second, a Couette flow applies a uniform shear across the
entire channel resulting in the same stress being applied to each cell in that channel – compared with a Poisseuille device, which applies no shear at the center and maximal shear at the walls or compared to a turbulent jet apparatus, which damages the cells via significant Reynolds stresses. Lastly, the Couette shear applied by the spinning rotor is independent of the axial flow moving fluid through the device. This means applied stress and exposure time can be investigated as independent variables unlike in Poisseuille devices where applied stress is a function of the pressure induced flowrate.

There are undoubtedly some disadvantages to investigating blood damage with a Couette shear device as opposed to other apparatus used in the field. The Couette device which is the subject of this thesis is mechanically complex and expensive to produce or replicate, as are many other similar devices in the literature. Selecting this particular shearing device design introduces some parameters which must be controlled and characterized such as latent electrical heat from the motor and magnetic bearings, rotor stability, Taylor vortices, surface finish, etc. That being said, such a device provides advantages that Poisseuille, jet-flow, and microfluidic devices cannot.

2.3 Development of the RIT Lev-VAD

A large portion of the total effort of this thesis is in the development of modifications to the original Lev-VAD so that it can be used to reliably apply simplified, controlled Couette shear to a fluid. Beyond this thesis’ particular investigation of blood damage, this shearing device can be used in future studies provided the device parameters, operation, and experiment procedure are well developed and documented. Such research with this device will also have special significance to further development of the parent RIT Lev-VAD
device. In short, the completion of this thesis will produce a well-developed experiment apparatus and procedure for the investigation of future blood damage and LVAD studies.

2.4 Objectives

The primary objective of this thesis is to experimentally characterize red blood cell damage as a function of applied fluid shear and exposure time in a rotational Couette flow, similar to that which is found in modern LVAD devices. Further, another objective is to evaluate and compare the extent of this damage in blood of multiple animal species, most significantly ovine and porcine blood. Throughout the pursuit of these specific objectives, an overlaying objective is to make useful observations about the fragility and mechanical behavior of blood in rotational shear flow to better contextualize existing blood damage literature.

To accomplish these objectives requires the construction a device and compatible experiment apparatus that is capable of doing so and reliable in its performance. This includes the modification of the internal structure of the Lev-VAD as well as appropriate cooling and protection of electronics from laboratory hazards. It also extends to the assembly, calibration, and validation of all necessary sensors to adequately monitor each critical experiment parameter as well as the iteration of a LabVIEW interface to manage and operate the experiment and the design of a MATLAB script to perform analysis of the acquired data over a large quantity of blood samples.
3 Methods – Test Apparatus and Experiment Parameters

3.1 Experiment and Apparatus Overview

The primary objective of this thesis is to evaluate hemolysis as a function of Couette shear stress and exposure time to that shear stress. In this apparatus, exposure time is a calculated value based on the flowrate and the geometry of the shearing region, as in Equation (3-1).

\[ t_{exp} = \frac{(Gap \ Length)(Cross \ Sectional \ Area)}{(Flow \ Rate)} = \frac{LA_{CS}}{Q} \]  

The applied shear rate is calculated as a function of the geometry of the shearing region and the rotor speed, as in Equation (3-2).

\[ \dot{\gamma} = \frac{\pi(Rotor \ Diameter)(Rotor \ Speed)}{(Shearing \ Gap \ Thickness)} = \frac{\pi D \omega}{a} \]  

The shear stress can then be determined as the product of shear rate and fluid viscosity, as in Equation (3-3).

\[ \tau = \dot{\gamma} \mu \]  

This approach allows fine control over the primary experiment parameters - fluid shear and exposure time - via adjustment of the rotor speed and flowrate respectively.

A syringe pump is used to control flowrate and push blood through the shearing device, after which it is collected as a drip from the outlet tubing into 2ml centrifuge tubes. There is a LabVIEW program which performs the bulk of data logging and processing. This
records temperatures from two thermocouples, line pressure upstream of the device, the rotor stability, the rotor speed, and requisite power – as well as several intermediate parameters. There is also a Boolean switch used to manually synchronize the collection of a sample to the data logging system. The physical apparatus, back-end schematic, and LabVIEW control panel are depicted in Figure 18, Figure 19, and Figure 20 respectively.

*Figure 18 – Schematic of experiment apparatus.*
Figure 19 - Schematic of apparatus back-end.

Figure 20 - Screen capture of LabVIEW front panel. This features orbit display with numerical quantification on the left, speed, stress, motor current, temperature and pressure on the right as well as data controls.
3.2 *Rotor Levitation and Stability*

The chief novel feature of this shearing device, as compared to other Couette shear devices that have been used in the literature, is the magnetic bearing system used to levitate the rotor. This offers an advantage in the uniform and symmetrical nature of the flow path as well as in the lack of conventional bearings which could be a locus for unquantified shear stress and secondary flow. The use of magnetic bearings does however introduce the stability of the rotor levitation as an experimental parameter.

As the rotational speed increases, the amount of rotor dynamic perturbation increases. There are two Hall Effect Sensor Arrays (HESAs), which measure this perturbation as voltages in the four Cartesian directions (positive/negative X and Y). One HESA does so from the front of the rotor and the other from the rear. These signals are fed into a PID control law in Simulink which applies power to the magnetic bearings to stabilize and levitate the rotor.

The spatial deviation of the rotor was measured in microns via high speed video and correlated to the signals given by the HESAs in volts. This validated the HESAs as an effective position measurement and allowed a calibration to convert from volts to microns.
Figure 21 - Still frame of high speed video with tracking plot. While the rotor was spinning, data was collected optically (blue) and via the HESAs (red). The close correlation of the signals indicates the validity of the HESAs.

In Figure 21, the still frame is taken from a camera pointed down the barrel of the device inlet. The red circle was used to calibrate the measured 14mm diameter to pixels. The white dot represents the tip of the rotor. The accompanying plot compares the motion tracked rotor tip with the position data collected by the Hall Effect sensors. The close agreement indicates the validity of the sensors for independent use going forward.

Proceeding, the signals given by the HESAs will be referred to as orbits, owing to the circular appearance of their trace on the XY plane as viewed axially.

While front and rear orbits were sufficient for monitoring the levitation of the blood pump this device is derived from, a critical dimension of this device is the thickness of the annular shearing gap, which is at the center of the rotor, between the two HESAs. In order to quantify variability in the gap thickness due to rotor deviation, an interpolation is performed on the two HESA signals to create a third, virtual orbit at the center of the rotor.
Chapter 3: Methods – Test Apparatus and Experiment Parameters

It is typical for the rotor to oscillate in a symmetrically conical fashion about the center. Therefore, the center orbit is always the smallest of the three.

![Diagram](image)

*Figure 22 - Diagram of HESA positions and method of interpolation. Depicted here is a radial offset and axial slant. This is atypical but allows a better visualization. Typically the orbit is conical about the rotor’s center.*

The standard deviation of the three orbits is used as a means of quantifying the uncertainty of the rotor position and is approximately equal to the radius of the orbit. The radius as given by this value typically lies between 10 and 20µm for the interpolated orbit (compared to a 127µm annular shearing gap width) but has been observed as high as 150µm at the front and rear (where the annular gap is 1.7mm). The deviation of the orbits gradually increases with rotor speed up to a critical speed around 7500RPM where it rapidly jumps to the maximum size observed. Further increasing the speed begins to shrink the orbits back down to typical levels. For the purposes of discussion in this work, the
regions below and above of the critical speed are referred to as Mode I and Mode II orbit respectively.

![Graph of orbit deviation vs rotor speed.](image)

*Figure 23 - Graph of orbit deviation vs rotor speed. These measurements were taken during a trial of porcine blood testing and are representative of the vast majority of observed orbit behaviors.*

![Images of LabVIEW orbit plots at different speeds in blood.](image)

*Figure 24 - Images of LabVIEW orbit plots at different speeds in blood. Note the dramatic leap in orbit size at super critical speeds. It remains centered but has a wide clearly defined trace.*

It is difficult to exactly translate the deviation of the orbits to an uncertainty in applied stress in a closed-form way. As the rotor moves off the device’s central axis by some amount, it correspondingly enlarges and shrinks the shearing gap on opposite sides of the rotor, which subsequently decreases and increases the applied shear rate in that instant. Due to the oscillating nature of this behavior, it has been assumed that the average
applied shear over the entire fluid cross section remains relatively constant. This also undoubtedly affects the flow regime in the device with respect to turbulence, Taylor vortices, and recirculation, which will be addressed in section 3.6.

![Diagram of stress magnitudes as rotor deviates from center. The narrowed gap width exerts a higher stress on blood in that region. This would occur in both X and Y at the front and rear in a conical fashion.](image)

**Figure 25** - Diagram of stress magnitudes as rotor deviates from center. The narrowed gap width exerts a higher stress on blood in that region. This would occur in both X and Y at the front and rear in a conical fashion.

### 3.3 Rotor Speed

Another critical parameter of the applied stress is the rotational speed. A brushless 3-phase motor in a delta configuration provides the torque to the rotor by alternating the magnetic polarity of each motor coil around the circumference of the device. This forces the rotor to continually rotate to re-align its interior permanent magnets with the stator electromagnetic coils.
Chapter 3: Methods – Test Apparatus and Experiment Parameters

The frequency of each motor phase is directly proportional to the rotational speed by a factor of 30.

\[
\omega_{\text{RPM}} = 30 \times f_{\text{Hz}}
\]  

By probing one phase and performing a Fourier transform on the resulting signal, the instantaneous speed can be logged via the live data acquisition LabVIEW program. The live speed measurement is then used as an input for a closed-loop speed control for the device in which the output motor control voltage is coerced to minimize the difference between desired and the measured speed. In this manner, the speed can be precisely controlled and the corresponding uncertainty recorded during an experiment. This technique was validated using a frequency strobe measuring physical rotation of the rotor.
3.4 Viscosity and Stress

Viscosity is the final term of the stress equation and is considerably less straightforward of a measurement for this device. In the literature, blood viscosity is typically measured at the start of an experiment at a relatively low shear rate. However this does not account for any shear-thinning, temperature, or Fahraeus-Lindqvist effect related changes in viscosity that may occur during testing.

Early testing of this device indicated that direct measurement of viscosity would provide much needed clarification of the applied stress. For this system, the viscosity of the fluid affects the required power to spin the rotor at a given speed. Higher viscosity fluids require higher torque from the motor. A characterization of torque is effectively a measurement of viscosity; this is the same principle standard cone-plate viscometers operate under. The torque, and by extension viscosity, can be represented as a function of the rotor speed and required electric current.

A calibration was performed in which fluids of known viscosity were pushed through the device and the applied current for different speeds measured. This was done with distilled water at 1cP and two viscosity calibration fluids at 5cP and 9cP at a high rate of flow to minimize any thermal influence on viscosity.
Figure 27 - Plot of viscosity calibration as measured between 3kRPM and 7.5kRPM. Dashed lines represent extrapolation of curves. An interpolated 3cP line is shown to demonstrate the linear quality of current as a function of viscosity for a given speed.

Three quadratic fits ($R^2=0.99$) were made for each fluid which made it clear that for a given speed, the viscosity is linearly proportional to motor current. Proceeding forward, an approximation of fluid viscosity can be made by interpolating between 5cP and 1cP curves for any instantaneously measured speed and current. This feature was added to the LabVIEW program. Much like a standard cone-plate viscometer, the mag-lev shearing device can only perform this measurement when the rotor is spinning.

With each of the critical parameters for calculating Couette stress being measured directly, it becomes possible to calculate the applied stress directly in real time as well as the subsequent random uncertainty. Further, a similar control law to that described above can be used to close the loop on stress instead of rotor speed.
3.5 Temperature

It has been noted above that there is a substantial amount of heat generated within the shearing device itself. Early on in this work, the generated heat was identified as a potential problem and an aluminum, actively-cooled, heatsink enclosure was designed to protect and prevent overheating of the electronics as well as to help mitigate the transfer of heat from the electrical components to the working fluid, in this case blood. The brushless motor contributes heat proportional to the power required to spin the rotor. This is likely also a factor in other experiments in the literature using similar means of torque. However, the active magnetic bearing system (AMB) is also a major source of heat which is unique to this device. The generated heat is proportional to the power required to stabilize the orbit, which, like motor power, also increases with rotational speed. In addition, the energized coils produce an inductive heat source in the passive magnets of the rotor which is also dissipated into the working fluid.

![Diagram of thermally significant features of device, **not to scale** (L). Model of aluminum heatsink enclosure (R).](image)

*Figure 28 – Diagram of thermally significant features of device, **not to scale** (L). Model of aluminum heatsink enclosure (R).*
The key thermal parameters involved are the temperature of the coolant, the flowrate of the working fluid, the power to the AMB’s (proportional to stability), and the power to the motor coil (proportional to speed). The motor power has been measured in blood to linearly increase from 5 to 30W over the entire range of rotor speeds possible whereas the AMB power ranges from 2 to 7W per bearing, of which there are 4, over the observed orbit sizes. Making the simplifying assumption that 100% of coil power is dissipated as heat into the working fluid, this sets a very conservative upper bound of heat as 60W for maximal heat conditions in blood. While the heatsink enclosure is certainly able to cool some of this, early testing in blood revealed that the cooled heatsink is inadequate to completely dissipate heat when operating at high rotor speeds and low flowrates, meaning high residency time in the device.

![Component Power Vs Rotor Speed in Blood (Porcine Trial #3)](image)

*Figure 29 – Plot of component power in a given blood experiment. Only one of four bearings’ power is conveniently measured at a given time, so the largest, FX, is used to approximate the power for all four. Taken from charge D of Porcine #3.*
In the experiment apparatus, temperature is logged with a calibrated K-type thermocouple at the inlet and outlet of the device as shown in Figure 18. Most combinations of speed and flowrate reach a steady state temperature difference of less than 5°C – especially when operating at speeds in Mode I orbit where AMB heat generation is low. However, the previously described high speed, high exposure time conditions have seen unreasonably higher temperature differences which are undoubtedly a factor in the resulting hemolysis in these samples. This led to several adjustments to trial methodology as well as additional filtration criteria for the acceptance of hemolysis data.

There is also a reasonable concern with the lack of interior temperature monitoring as there is a possibility that the working fluid is heated by the bearings but is subsequently cooled in the outlet just before reaching the monitoring thermocouple. An auxiliary experiment was performed in order to better characterize the internal temperature. All other parameters being held constant, blood viscosity can be described exclusively as a function of temperature. With the rotor acting as a Couette viscometer as described in section 3.4, blood pools of several initial temperatures were pumped through the device at a high rate of flow to ensure minimal differential temperature changes. The viscosity at each nominal temperature was recorded along with the upper and lower bound observed by the inlet and outlet thermocouples.
The resulting trend from this experiment cannot necessarily be used to exactly predict the internal device temperature (there are many other non-constant parameters affecting viscosity); it does allow for an approximation to be made for the purposes of conservatively screening out invalid hemolysis data and suggests there is no significant cooling from the internal of the device to the outlet. In other words, the recorded outlet temperature is almost certainly at, or within a few degrees of, the maximum temperature of the system.

3.6 Fluid Flow Analysis

As with any experiment studying fluid mechanics, it is necessary to fully describe the fluid flows in the system. In this case, there is an axial flow component throughout the system which is analyzed as a Poissoeuille flow, as well as the Taylor-Couette flow within the device itself. The three especially relevant parameters to this experiment are the Reynolds number - to characterize turbulence, the Taylor number - to characterize vortices and recirculation, and the residency time. The particular regions of interest are defined as the
annular shearing gap region within the device, the non-gap region within the device, and the 1/16” tubing in the rest of the apparatus. For the sake of analysis in this section, blood is assumed to have a viscosity of 3.6cP and 1.06g/ml.

The simplest component of flow in the system is the axial Poiseuille flow through the annulus of the rotor and housing tube and through the circular cross-section of the apparatus tubing. The volumetric flowrate is the tuning parameter for testing different exposure times. As such it is important to analyze and acknowledge how changing flowrate affects the axial flow component throughout the system.

<table>
<thead>
<tr>
<th>Flow Rate [ml/min]</th>
<th>Exposure Time [ms]</th>
<th>Device Residency Time [s]</th>
<th>Shearing Gap Region</th>
<th>Non-Gap Region</th>
<th>Tubing Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>1150</td>
<td>65</td>
<td>1.17</td>
<td>0.44</td>
<td>0.10</td>
</tr>
<tr>
<td>6.3</td>
<td>1000</td>
<td>57</td>
<td>1.34</td>
<td>0.50</td>
<td>0.11</td>
</tr>
<tr>
<td>7.4</td>
<td>850</td>
<td>49</td>
<td>1.58</td>
<td>0.59</td>
<td>0.13</td>
</tr>
<tr>
<td>9.0</td>
<td>700</td>
<td>40</td>
<td>1.92</td>
<td>0.72</td>
<td>0.16</td>
</tr>
<tr>
<td>11.5</td>
<td>550</td>
<td>31</td>
<td>2.45</td>
<td>0.92</td>
<td>0.20</td>
</tr>
<tr>
<td>15.8</td>
<td>400</td>
<td>23</td>
<td>3.36</td>
<td>1.26</td>
<td>0.28</td>
</tr>
<tr>
<td>25.2</td>
<td>250</td>
<td>14</td>
<td>5.36</td>
<td>2.01</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Table 1 – Table of axial flow parameters in experimental system.

Table 1 reviews the range of flowrates needed to cover the range of exposure times under investigation. Assuming no recirculation caused by non-axial flow components, blood residency times range from 14 seconds to 1 minute. Given that axial Reynolds numbers in all three regions are extremely low, it is unlikely that there is significant mixing occurring at any point in the system due to axial flow components.

A more complicated flow component exists within the device in the circumferential direction of the annulus of the rotor and housing tube. In this thesis, this flow will be modeled as a Taylor-Couette flow, described in section 1.2. The two key questions to ask
with respect to this flow component are: is the flow in a given region turbulent, and are there Taylor vortices present there? There is a considerable degree of deeper nuance to be explored in these two questions, however to do so is difficult to accomplish analytically and to take another approach is time consuming and beyond the scope of this work.

This analysis makes use of two disagreeing methods of predicting the presence of vortices from the literature. The first is a comparison between Taylor number and critical Taylor number as given by a formula derived by Schwarz et al. (1964). This is based on experimental observation of a Taylor-Couette flow with an axial through-flow, stationary outer cylinder, and narrow gap annulus (radius ratio \( \eta = 0.95 \)); this is an almost exact description of the system being analyzed, especially the narrow shearing gap region. In that work, the critical Taylor number was found to have parabolic dependence on axial flowrate and below an axial Reynolds number of 25, describable by Equation (3-5) [52].

\[
Ta_{cr}^2 = \frac{2 \left( \frac{R_1}{R_2} \right)^2 (R_2 - R_1)^4 \Omega^2}{1 - \left( \frac{R_1}{R_2} \right)^2 \frac{v^2}{\nu^2}} ; \quad Re_{Axial} < 25
\]  

(3-5)

The second method is given by Andereck et al. (1986) which is derived from the observation of independently rotating concentric cylinders, \( \eta = 0.833 \). The rich flow structures were described as a function of the Reynolds numbers given by the outer and inner cylinder surface velocities. In the case of this thesis, the outer Reynolds number is 0. Figure 5 in section 1.2 is a chart of these rich flow structures taken from Andereck (1986) and reproduced in color by Grossman et al. (2016) [9], [11].
Table 2 – Table of Taylor-Couette flow parameters in annular shearing gap region of device.

<table>
<thead>
<tr>
<th>Speed (RPM)</th>
<th>Rad/s</th>
<th>Stress (Pa)</th>
<th>Re_{Rotational}</th>
<th>Ta_{Rotational}</th>
<th>Ta_{Crit} (Schwarz)</th>
<th>Vortices Present? (Schwarz)</th>
<th>Flow Type (Andereck)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,000</td>
<td>314</td>
<td>87</td>
<td>115</td>
<td>171</td>
<td>1,303</td>
<td>No</td>
<td>Couette</td>
</tr>
<tr>
<td>5,000</td>
<td>524</td>
<td>145</td>
<td>191</td>
<td>475</td>
<td>2,171</td>
<td>No</td>
<td>Wavy Vortex</td>
</tr>
<tr>
<td>7,000</td>
<td>733</td>
<td>203</td>
<td>267</td>
<td>930</td>
<td>3,040</td>
<td>No</td>
<td>Wavy Vortex</td>
</tr>
<tr>
<td>9,000</td>
<td>942</td>
<td>260</td>
<td>344</td>
<td>1,538</td>
<td>3,909</td>
<td>No</td>
<td>Wavy Vortex</td>
</tr>
<tr>
<td>11,000</td>
<td>1,152</td>
<td>318</td>
<td>420</td>
<td>2,927</td>
<td>4,777</td>
<td>No</td>
<td>Wavy Vortex</td>
</tr>
</tbody>
</table>

Table 3 – Table of Taylor Couette flow parameters in annular non-gap region of device.

<table>
<thead>
<tr>
<th>Speed (RPM)</th>
<th>Rad/s</th>
<th>Stress (Pa)</th>
<th>Re_{Rotational}</th>
<th>Ta_{Rotational}</th>
<th>Ta_{Crit} (Schwarz)</th>
<th>Vortices Present? (Schwarz)</th>
<th>Flow Type (Andereck)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,000</td>
<td>314</td>
<td>6</td>
<td>1,275</td>
<td>333,986</td>
<td>55,034</td>
<td>Yes</td>
<td>Modular Waves</td>
</tr>
<tr>
<td>5,000</td>
<td>524</td>
<td>9</td>
<td>2,125</td>
<td>927,740</td>
<td>91,724</td>
<td>Yes</td>
<td>Turbulent Taylor Vortex</td>
</tr>
<tr>
<td>7,000</td>
<td>733</td>
<td>13</td>
<td>2,975</td>
<td>1,818,370</td>
<td>128,413</td>
<td>Yes</td>
<td>Turbulent Taylor Vortex</td>
</tr>
<tr>
<td>9,000</td>
<td>942</td>
<td>17</td>
<td>3,825</td>
<td>3,005,877</td>
<td>165,103</td>
<td>Yes</td>
<td>Turbulent Taylor Vortex</td>
</tr>
<tr>
<td>11,000</td>
<td>1,152</td>
<td>20</td>
<td>4,675</td>
<td>4,490,261</td>
<td>201,792</td>
<td>Yes</td>
<td>Turbulent Taylor Vortex</td>
</tr>
</tbody>
</table>

Table 2 and Table 3 reviews the range of rotor speeds required to cover the range of shear stresses under investigation, each pertaining to the shearing gap region and non-gap region respectively. Both of the above described methods are shown with contradictory results. The Schwarz critical Taylor number predicts that there are no Taylor vortices present within the shearing gap region but that there almost certainly are in the non-gap region. The Andereck flow types, predicted based on the rotor’s rotational Reynolds number, are wavy vortex in the shearing gap region and turbulent Taylor vortices in the non-gap region. The Andereck approach does not account for any axial through-flow effects which is certainly present in the flow structures under analysis. However, the Schwarz method was only validated for a narrow gap geometry, which certainly describes the shearing region but the non-gap region is slightly larger than what was studied in Schwarz (1964).
To further complicate the analysis, it is possible, and indeed likely, that the vibrations caused by the rotor’s spin and orbit introduce some degree of turbidity and lower the critical Reynolds and Taylor numbers by some amount. It is also true that viscosity is not constant throughout the system, changing with shear rate, temperature, and potentially channel size as predicted by the Fahraeus-Lindqvist effect. It is nearly impossible to totally describe the flow structures of this system via analytical techniques based on evidence from other studies examining different geometries or not considering axial flow influence. Given the above analysis, this thesis will proceed under the assumption that there is no turbulence or Taylor vortices within the shearing gap region, but that there are vortices present in the non-gap region which could even be turbulent. Therefore, the presence of these flow structures will not likely influence the primary experiment variables of applied stress and exposure time, though they may increase device residency time by some un-quantified factor or have other effects on secondary experiment parameters. Further exploration of this topic will be recommended in section 6.3 as future work.

3.7 Honing and Polishing

Both the rotor and housing tube were polished but no reliable measurements of surface roughness have been performed. The stainless steel rotor was first polished with 80, 150, and 220 grit sandpaper before progressing to a 400 and 800 grit lapping compound on a felt Dremel bit. The Delrin plastic sleeve was left unpolished from the finish it was machined at. The stainless steel housing tube was polished first with 60 then 120 grit Flex-Hone tools to assure even radial pressure and then with 400 and 800 grit lapping
compound applied to a polishing paper. These grits likely correspond to an average surface roughness of approximately $1\mu$m or less [53]–[55].

### 3.8 Active Magnetic Bearing Gain Tuning

The magnetic bearing system operates under a Proportional-Integral-Differential (PID) control law with gains governing each element. In the current configuration of the device’s Simulink operating software, the P and I gains are easily tunable for both the front and rear. Higher gains allow the control law to apply more effort toward maintaining rotor levitation. This in turn can mean more energy in the bearing coils which equates to more heat entering the system. There is also the potential for overshoot by the control law which must then be subsequently corrected, wastefully introducing even more heat. It follows then, that the appropriate selection of the bearing gains is necessary to minimize heat and to optimize rotor stability. This optimization is a complicated task because of the complex viscoelastic properties of blood, which in this system act as an additional fluid bearing, stabilizing the rotor and reducing the need for the electromagnetic bearings to be energized.

A set of gains was arrived at via empirical testing with an emphasis placed on minimizing orbit size over the entire range of rotor speeds under investigation. This of course results in higher energy and the thermal effects described in section 3.5. It may also be related to the observed behavior of the rotor orbits when operating at, and on either side of, the critical rotor speed described in section 3.2. That is to say, gains set too high could cause overshooting which allows the extremely large conical orbit to develop immediately above the critical rotor speed.
4 Methods – Blood Damage Experiment

4.1 Blood Sourcing and Preparation

All blood used for final hemolysis testing was purchased as whole blood from Lampire Biological Laboratories (Pipersville, Pennsylvania) in 1 liter quantities. The standard bleed site is from the jugular vein after application of a tourniquet and topical disinfectant. Blood is allowed to flow through a needle (14g for ovine, 16g for porcine) into a sterile bottle prepared with 15:85 ratio of ACD anticoagulant to blood. Each order was shipped on ice overnight for hemolysis testing 1 day after draw.

Upon arrival to the lab, the blood is filtered through a polypropylene mesh screen with 100µm pores. All blood contacting bottles and beakers are first rinsed with phosphate buffered saline (PBS). After filtration, a 2ml sample is removed to assess background levels of hemolysis on arrival. The hematocrit of the pool is measured via glass micro-capillary tube assay and in most trials was adjusted to 36%.

In total, 8 trials of blood were conducted – 4 in ovine blood and 4 in porcine blood in order to better demonstrate reproducibility and variability between donors of a single species. It is unclear how the sex, age, or breed of the donor animal affects red cell fragility as this is not typically discussed in comparable studies in literature. As such, no effort was made to control these parameters, although they have been recorded here for reference.
Chapter 4: Methods – Blood Damage Experiment

4.2 Experiment Procedure

Prior to testing, the water chiller is set to 10°C and run for 1 hour to allow the entire device-heatsink enclosure system to reach an equivalent steady state. First, the entire apparatus depicted in Figure 18 is purged through with PBS and all tubing and fittings are manually manipulated and struck to force all bubbles out of the system.

Each blood trial is broken up into approximately 15 “charges” of 55ml drawn into a 60ml syringe at a constant flowrate. Prior to loading the charge, the blood pool is gently but thoroughly mixed and a background sample is drawn. Each charge is pushed by the syringe pump through the shearing device in one shot at a single flowrate with no starting or stopping of the syringe pump. Typically, twenty-two 2ml centrifuge tubes are lined up at the outlet of the fluid system. Once the syringe pump begins infusion, the first 12ml of blood are purged into a waste beaker. For reference, the volume of the entire system is 8ml (6ml for just the device). After the purge, samples are dripped into the prepared tubes, continuously moving from one tube to the next, with the LabVIEW collection Boolean being cycled by a second operator to synchronize the data log to each sample. Testing is conducted with the blood at room temperature (approx. 23°C), measured directly by the

<table>
<thead>
<tr>
<th>Trial</th>
<th>Test Date</th>
<th>Test Hematocrit</th>
<th>Donor Sex</th>
<th>Donor Age</th>
<th>Donor Breed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovine #1</td>
<td>3/6/2018</td>
<td>33</td>
<td>F</td>
<td>5 Years</td>
<td>Suffolk Cross</td>
</tr>
<tr>
<td>Ovine #2</td>
<td>6/6/2018</td>
<td>25</td>
<td>F</td>
<td>7 Years</td>
<td>Suffolk/Dorset</td>
</tr>
<tr>
<td>Ovine #3</td>
<td>6/13/2018</td>
<td>36.0</td>
<td>F</td>
<td>1 Year</td>
<td>Merino</td>
</tr>
<tr>
<td>Ovine #4</td>
<td>6/21/2018</td>
<td>32.5</td>
<td>F</td>
<td>2 Years</td>
<td>Hamp/Suffolk Cross</td>
</tr>
<tr>
<td>Porcine #1</td>
<td>7/3/2018</td>
<td>36.3</td>
<td>F</td>
<td>4 Years</td>
<td>Yorkshire Cross</td>
</tr>
<tr>
<td>Porcine #2</td>
<td>7/10/2018</td>
<td>36.3</td>
<td>F</td>
<td>4 Years</td>
<td>Yorkshire Cross</td>
</tr>
<tr>
<td>Porcine #3</td>
<td>8/2/2018</td>
<td>36.0</td>
<td>F</td>
<td>5 Years</td>
<td>Yorkshire Cross</td>
</tr>
<tr>
<td>Porcine #4</td>
<td>8/9/2018</td>
<td>34.1</td>
<td>F</td>
<td>8 Years</td>
<td>Yorkshire Cross</td>
</tr>
</tbody>
</table>

Table 4 – Table of trials and donor information.
inlet thermocouple. In the event that the outlet temperature rises above 35°C, the device is powered off for the remainder of the charge, though sample collection continues. This is done as a precaution against any more extreme temperatures in the system. After the full charge has finished, an additional 12ml of blood is withdrawn and purged through the device. This is let to stand for 10 minutes to allow the motor and bearing coils to cool down. This would not be necessary for the electronics, but it ensures that each charge begins at the same thermal conditions. The same procedure is then repeated for the subsequent charge, beginning with the mixing of the pool and sampling of background levels.

![Figure 31 - Photos of blood shearing experiment. The rotor and electronics are housed in the aluminum block depicted on the left. Flow runs vertically upward to ensure no air pockets are trapped within. The syringe pump applies a constant flow of blood which is collected into 2ml sampling tubes.](image)

The typical sampling strategy of a trial is to conduct 7 charges which are upward sweeps from minimum to maximum rotor speed at 7 different flowrates which correspond
to an even coverage of exposure times. Additional auxiliary charges are used to conduct spot checks, alternative sweep configurations, or otherwise investigate the significance of other parameters. Each trial day, 1 charge is dedicated to a dynamic control, wherein the rotor is levitating but not spinning. This is intended to capture any damage caused simply by contact with the system or by blood handling before and after collection.

All collected samples are centrifuged at 3,000G after which the resulting plasma is pipetted off into a fresh tube. These plasma-only tubes are then spun once more at 13,000G to reveal a small pellet of red cells at the bottom of the tube. This method ensures that no red cells are mistaken for plasma free hemoglobin in absorbance testing. Each sample is plated into a 96-well plate with 200uL of plasma per well and one well per sample. Two wells are filled the same with PBS as an absorbance control with an equivalent optical path-length.

![Figure 32 – Example of plate from Porcine #2 showing low to high damage range. Note the range of plasma colors as compared to the two PBS “blank” wells shown in the bottom right.](image)
The plates are then measured for absorbance at 560nm, 576.5nm, and 593nm wavelengths for later application of Cripps Method hemolysis assay. Wherever absorbance is measured above 2.0, the sample is re-plated at 25% dilution into PBS in order to avoid the plate reader's upper sensing limit. Each plate is read twice for verification of measurements.

4.3 Data Analysis – Sample Averaging

With the samples being collected continuously and the device parameters changing as they are, it is necessary to have a robust methodology of averaging these parameters for the duration of the collected sample. Because it takes time for a sample to flow from the shearing gap to the outlet, the synchronization marker given by the collection Boolean must be adjusted backward in time to account for this delay. It is possible and indeed likely that there is some degree of mixing within the housing tube due to the complex flow structures described in section 3.6. To account for this, when the parameters of a sample are averaged, they are averaged over the “fluid column's” entire residency time within the device. In the device, a column with such rigidly defined boundaries as displayed in Figure 33 does not likely exist. However, it is useful to model it as such for analytical and descriptive purposes and it is reasonable to assume that the true nature of the sample volume as it passes through the system behaves in a similar manner.
Chapter 4: Methods – Blood Damage Experiment

Figure 33 – Diagram of fluid column in experiment system.

Figure 34 – Graph demonstrating action of collection Boolean and resulting adjustment in “averaging time” for a sample.
Examining Figure 33 and Figure 34, the collection Boolean marks when {Fluid 1} crosses {Position C} until {Fluid 2} crosses {Position C}. This interval is then backed out in time ($t_1$ seconds) on the data log until it reaches the point at which {Fluid 1} crossed {Position A}. The device parameters are averaged from this point until {Fluid 2} crosses {Position B} (a period of $t_2$ seconds). Where:

$$t_1 = \left( \frac{V_{Tubing} + V_{Measurement}}{Flowrate} \right)$$  \hspace{1cm} (4-1)

$$t_2 = \left( \frac{V_{Measurement}}{Flowrate} \right)$$  \hspace{1cm} (4-2)

Put more simply, the Boolean interval is adjusted backwards in time, and then dilated in length. The adjustment ensures that the averaged device parameters per sample are representative of what they would have been when the sample was within the device itself, rather than at the outlet. The dilation ensures that each sample is consistently and conservatively bounded. If any mixing or recirculation occurs as a result of complex flow structures, the entire range of experienced stresses for a collected sample over the time it resided in the device is captured. Because the device volume is 6ml and the samples are each 2ml, the dilation is typically a factor of 3. And, for the same reason, there are often as many as 4 sample windows which overlap.
Figure 35 – Plot of a typical sweep charge showing overlapping sample windows in a transparent purple with the first, middle, and last sample outlined in red for emphasis of the window size. This plot is taken from Charge H of Porcine #1.
Figure 36 – Box-whisker plot of all samples from Charge H of Porcine #1 demonstrating the quantitative result of the overlapping averaging windows depicted in Figure 35.

Figure 35 and Figure 36 show the intermediate and final result of the described sample averaging methodology. In Figure 35, the averaging window for each consecutive sample is shown with purple shading which is slightly transparent to show the overlapping windows. Over the first four samples, the shading gradually gets darker as their windows overlap. The red outlines show the true size of a given sample window in this charge. Figure 36 shows this overlap more quantitatively on a box-whisker plot, sample-by-sample. While this is effective for validating the averaging methodology, it becomes difficult to show data as box-whisker points on 3-D axes of stress, exposure and hemolysis. For this reason, all final hemolysis data points are reported as the mean value of the averaging window with the range taken as the uncertainty bounds.
4.4 Data Analysis – Hemolysis

The raw absorption data is taken from the plate reader and the background levels measured in the PBS wells are subtracted from those containing plasma. Using Cripps method of absorption, the free hemoglobin is calculated in milligrams-per-deciliter.

\[
fHb = K \left[ A_{576.5} - \left( \frac{A_{560} + A_{593}}{2} \right) \right] \quad \text{where } K = 177.6
\]  
(4-3)

In order to normalize for background levels of damage, which were observed to gradually increase throughout the days testing, the background level of each charge (taken before it was loaded) is subtracted from the levels measured for the samples of that charge. It is also useful to report the level of hemolysis in terms of the index of hemolysis, in order to normalize observed damage between trials and other studies. Because equipment was not available to measure the total blood hemoglobin content, this was approximated as 1/3 the hematocrit as is supported in literature [29].

\[
IH\% = \left[ \frac{1 - \frac{Hct}{100} \cdot \left( \frac{fHb}{1000} \right)}{\frac{1}{3} Hct} \right] \times 100
\]  
(4-4)
5 Results

5.1 Primary Hemolysis Testing

This section will primarily consist of plots with no interpretation given and only brief explanation of what is represented. Section 5.3 describes auxiliary experiments which were conducted in an effort to answer many of the questions that arose during analysis of the primary hemolysis results presented here. Finally, a comparative presentation of results in the context of other works in the field and a broader discussion of the validity of findings are reserved for chapter 1.

All data represented here has passed the following acceptability criteria:

- Stress Uncertainty < 50Pa
- Outlet Temperature < 35°C
- Center Orbit < 30µm

In the majority of cases, special attention was paid to maintain scale consistency between figures for more convenient comparison, even where the measured “signal” was low.

As expected and as observed in literature, hemolysis has a direct positive correlation with both applied stress and exposure time to that stress. In both species, ovine and porcine, observed hemolysis was under 0.2% below 175Pa across all exposure times. Above this, the maximum observed damage was no more than 1%, occurring in porcine blood. For identical conditions, porcine blood trials yielded higher levels of hemolysis than ovine blood. In ovine blood, virtually no hemolysis was observed below 200Pa and only
slightly above 0.2% at the maximum. However, above 250Pa, a curious and counter-intuitive decrease in hemolysis was observed.

In Figure 37 through Figure 46, stress is given based on viscosity as it was measured in real time in the device and following the adjustment and averaging procedure described in section 4.3. As a point of reference however, a rotor speed of 7000RPM corresponds to a stress of approximately 200Pa assuming a constant blood viscosity of 3.6cP. This means that the observed threshold for damage is notably close to the critical rotor speed described in section 3.2.

Because of some irregularities that can be observed in plots depicting all valid hemolysis samples (Figure 38 and Figure 40) the subsequent plots in this section (Figure 41 through Figure 46) portray only experiment charges which consisted of an upward sweep in rotor speed across the entire capable range in order to allow for better comparison between species. These upward sweeps are henceforth referred to as the primary hemolysis data. The irregularities arise from auxiliary experiment charges such as constant rotor speed “spot checks” and downward sweeps in rotor speed. These are examined in greater depth in section 5.3.
Figure 37 – Plot of observed range during all ovine blood trials.

Figure 38 – Plot of observed hemolysis in all ovine blood trials. Color gradient indicates variable exposure time.
Chapter 5: Results

Figure 39 – Plot of observed range during all porcine blood trials.

Figure 40 - Plot of observed hemolysis in all porcine blood trials. Color gradient indicates variable exposure time.
Figure 41 - Plot of only upward sweeps in ovine blood trials. Color gradient indicates variable exposure time.

Figure 42 - Plot of only upward sweeps in porcine blood trials. Color gradient indicates variable exposure time.
Figure 43 – Plot of only upward sweeps of rotor speed for ovine and porcine blood.

Figure 44 – 3D plot of only upward sweeps of rotor speed for ovine and porcine blood.
Figure 45 – Upward sweeps from ovine trials #3 and #4, colored by donor.

Figure 46 – Upward sweeps from porcine trials #1-3, colored by donor.
5.2 Uncertainty Analysis

For the majority of measurements in this experiment, uncertainties are quantified as the standard deviation of the sample window multiplied by 2, thus theoretically capturing 95% of the possible values assuming a Gaussian distribution. While this assumption may not always hold, this method has been found to adequately capture the recorded range of values in virtually all cases. Owing to its ease of application, it is the ideal option for the majority of parameters. In addition to the random uncertainty, every parameter also has an associated systematic uncertainty which is a function of the equipment and tools used to perform measurements. For nearly every electronically monitored parameter however, the systematic uncertainty is significantly smaller than that of the random and thus assumed to be zero.

In the case of the most critical parameters, namely stress, exposure time, and hemolysis, an alternative approach is taken. Over multiple spectrophotometric readings of the same hemolysis plate, typical free hemoglobin variability was quantified at 5% error. This is carried through the index of hemolysis equation assuming no significant error in the measurement of hematocrit. As described in section 4.3, stress error is recorded both as two standard deviations as well as the upper and lower bound of recorded values in the averaging window. The former is used to screen out samples containing extremely high variability and the latter is used for a more accurate representation of uncertainty, shown in Figure 47. It is also worth considering that stress uncertainty could be described as a function of the rotor orbit, as the shearing gap annulus geometry is used in stress
calculation. As was shown in Figure 25, the orbit deviation is assumed to have a net zero effect on applied stress.

![Hemolysis Uncertainty Analysis in Porcine #2](image)

*Figure 47 – Plot depicting stress vs hemolysis with uncertainties for three charges of porcine trial #2.*

As the only parameter without a performed measurement associated with it, it is especially important the systematic uncertainty in flowrate be well characterized and minimized as it is the chief factor in exposure time uncertainty. The syringe pump was calibrated gravimetrically and found to have a variability described by:

\[
\Delta Q = 0.0023 Q^2 + 0.0284Q
\]  

(5-1)

This is then combined with the associated uncertainties from each other factor of exposure time in a route-sum-square formulation as shown in Equation (5-2).
\[
\Delta_{\text{Exp}} = \sqrt{\left(\frac{\partial Q}{\partial \text{Exp}}\right)^2 \Delta Q^2 + \left(\frac{\partial a}{\partial \text{Exp}}\right)^2 \Delta a^2 + \left(\frac{\partial CSA}{\partial \text{Exp}}\right)^2 \Delta_{CSA}^2} \tag{5-2}
\]

This formula gives a reliable assessment of the uncertainty of exposure time which is a function of the flowrate, as shown in Figure 48.

![Exposure Time Uncertainty Analysis in Porcine #2](image)

*Figure 48 – Plot depicting uncertainty in exposure time for porcine trial #2.*

### 5.3 Auxiliary Testing

In addition to the quantitative numerical uncertainties pertaining to measurement techniques described in section 5.2, there are also a number of characteristics of the device and test methodology which could potentially reduce the fidelity of the primary hemolysis results reported in section 5.1. This section describes several auxiliary experiments which were conducted with the intent of identifying and exploring possible points of criticism of
the device and methodology. Also documented are a number of observations made during
the span of testing which additionally explain particular aspects of this thesis and
otherwise better inform future works.

5.3.1  High Hematocrit Comparison

Because there was some variability in test hematocrit between trials, especially in
the ovine trials, it was necessary to perform some testing to understand the effect of blood
hematocrit on the observed hemolysis in this experiment. During porcine trials #3 and #4
(primarily tested at 36.0 and 34.0) a separate pools of blood at higher and lower
hematocrits were reserved and upward sweeps were performed at two exposure times,
replicating exactly the primary testing but at higher and lower hematocrits. The hypothesis
was that the higher hematocrit pool would yield proportionally higher plasma free
hemoglobin than the lower hematocrit pool. Not only are there more cells present to be
lysed, but the bulk viscosity is higher in the higher hematocrit pool as well, leading to
higher applied shear stress at the same shear rate. The higher level of damage would then
be compensated down by the index of hemolysis equation to result in the same percentage
of damage between the two hematocrit levels as a function of applied stress. The same line
of thought applies for low hematocrit, though oppositely. Interestingly, this was not what
occurred. Due to the increased bulk viscosity in the high hematocrit pools, much higher
calculated stresses were applied over the same range of shear rates and in the low
hematocrit pools, much lower stresses. However, nearly identical levels of hemolysis,
regardless of hematocrit, occurred as a function of shear rate, as shown in Figure 49 and
Figure 50.
Figure 49 – Plot of hemolysis as a function of applied stress at different hematocrits. Viscosity is a factor in this representation.

Figure 50 – Plot of hemolysis as a function of applied shear at different hematocrits. Viscosity is not a factor in this representation.
Two possible explanations are immediately obvious. The first is that the bulk, whole blood viscosity is, in fact, irrelevant to shear-induced blood damage in this experiment and at large. While it may affect the flow structures in this device and in other Couette-shear devices in literature, the increased bulk viscosity of blood due to increased hematocrit may not effectively increase the degree of strain experienced by a cell at a given shear rate and so does not lead to increased lysis. It should be noted however, that the bulk blood viscosity is not necessarily equivalent to the viscosity of the suspension medium, namely the plasma. It may be that changes in plasma viscosity would have a measurable effect on the resulting damage at a given shear rate. Regardless, all results figures displayed in this chapter have been reproduced as a function of shear rate rather than stress in order to represent the data set in non-bulk viscosity dependent terms. The reproduced figures can be found in Appendix A: Reproduced Results in Terms of Shear Rate.

The other potential explanation for the observed phenomenon is that over the examined range of shear in this device specifically, the shear applied by the gap region does not significantly damage blood and the observed hemolysis across all trials and tests is exclusively a result of secondary damage factors which are directly correlated with high shear rate such as increased temperature and complex flow structures. While this auxiliary experiment consists of nine charges worth of data, it is advisable that this area be further studied due to its high significance to the interpretation of this experiment and to the blood damage field at large. Further study is strongly recommended in section 6.3.
5.3.2 Low Gain Comparison

Towards the end of the study, lower gains were experimented with in an attempt to minimize bearing energy to evaluate the effect the hypothetically lowered temperature has on observed hemolysis. The lower gains can allow the rotor to swing wildly in a non-circular fashion when at sub-critical rotor speeds. This does not always occur however the intermittent stability when operating at low gains in the sub-critical Mode I orbit region is precisely the reason higher gains have been used until this point. In the super-critical Mode II orbit region, lower gains moderately lower the power consumed by the bearings and virtually eliminate the jump discontinuity that occurs at the critical rotor speed.

![Comparison of High to Low Gain Power Consumption (Porcine Trial #3)](image)

*Figure 51 – Plot of the bearing power associated with different gain settings. Note the low-gain oscillations in the low range of speeds and the lack of power spike at the critical rotor speed.*

Several upward stress sweeps in porcine trials 3 and 4 were conducted at both low and high gain settings over a range of exposure times and the hemolysis was compared. Surprisingly, recorded temperatures at the low gain settings were not significantly different from those at high gain settings. Despite this, levels of hemolysis were much lower in the low gain sweeps, shown in Figure 52 and Figure 54. In addition, the measured viscosity was higher in the low gain sweeps. There is no obvious reason for the viscosity to be different as the hematocrit, temperature, and shear were equivalent. This suggests that the lower gain settings affected the current drawn by the motor at any given speed,
compromising the viscosity measuring technique described in section 3.4. For this reason, the measured hemolysis has also been represented against shear rate in Figure 53 and Figure 55. When plotted against shear rate, the discrepancy remains for each condition, curiously, save for the highest exposure time in porcine trial 3.

Figure 52 – Plot of hemolysis during upward sweep tests at different gain settings as a function of stress (P#3).
Figure 53 – Plot of hemolysis during upward sweep tests at different gain settings as a function of shear rate (P#3).

Figure 54 - Plot of hemolysis during upward sweep tests at different gain settings as a function of stress (P#4).
Figure 55 – Plot of hemolysis during upward sweep tests at different gain settings as a function of shear rate (P#4).
Figure 56 – Plot of all upward porcine sweeps separated by low and high gain setting. Red: Primary hemolysis sweeps at high gain, Blue: Low gain sweeps.

Investigating every other declared experiment parameter, no difference was found between high and low gain sweeps save for their orbit profile as a function of speed, shown in Figure 57.

Figure 57 – Plots of rotor orbits at different gain settings. Note the lack of discontinuity in low gains at the critical rotor speed.
The observed deviation in damage between gain settings begins at \( \sim 48,000 \text{s}^{-1} \), corresponding to approximately 6000RPM in this case. It is possible that the larger orbits present in the Mode II region at high gain settings and the sharp increase in orbit size generates some turbulence and accompanying Reynolds stress which lyses cells. However, 6000RPM is well below the critical rotor speed and the orbit profile in the Mode I region is fairly similar between low and high gain. Given that such a noticeable and consistent difference is present between the settings, but no explanation is obvious, more testing is advisable before drawing firm conclusions about the origin of this difference.

### 5.3.3 Constant Stress Spot Checks

Throughout primary testing, it became clear that the significant thermal contributions of the motor and bearing electronics have a non-negligible effect on red cell fragility and observed hemolysis. In an effort to better comprehend the extent of this influence, several experiment charges were devoted to “spot-check” constant shear rates and evaluate hemolysis as compared to measured values for an equivalent portion of an upward sweep. These charges are notable in Figure 40 as nearly continuous vertical lines of increasing damage.

Four spot-check sweeps are presented in Figure 58 taken at 150Pa and 190Pa over the entire range of exposure times. Despite a nearly constant shear stress, an increase in hemolysis was observed over time for the 190Pa charges at medium and long exposure times. One possible explanation for this is that there is some time constant associated with the progression of sheared blood from the shearing gap to the outlet of the system. That is to say, there is a lag between when a shear stress is applied and when the outlet blood
becomes representative of that degree of damage, more so than is captured by the
adjustment and dilation procedure described in section 4.3. If that time constant were the
case however, its effect would almost certainly be observable in the other charges, one at
the same stress but shorter exposure time and two others at only 40Pa less at the same
exposures. In those charges however, there is no increase in hemolysis over time or even
any significantly measurable above background levels. This supports the conclusion that
any system time constant that exists is adequately captured by the adjustment and dilation
procedure and does not impact results observed in primary testing.

The next most obvious explanation for this phenomenon is that the dramatic
increases in temperature over time measured in the two anomalous charges alters the
fragility of the cells or directly damages them outright. Because 190Pa presses close to the
lower side of the critical rotor speed, energy to the magnetic bearing coils is elevated and
results in increased heat. While this has little effect on blood which is only exposed for a
short period, the medium and long exposure time charges are more significantly impacted.
Every other parameter including orbit deviation was examined for correlation and none
was found as strong as that between temperature and hemolysis.
Figure 58 – Sample-by-sample plots of Constant shear charges from porcine trial #2. As a supplement, the observed range of damage from primary sweep testing in equivalent ranges is shown on the right.
5.3.4 Upward Sweep vs Downward Sweep

In order to further investigate the possibility of a collection time constant or thermal effect on observed damage, several sweeps were performed in reverse of the typical upward sweep that composes the primary hemolysis testing data. The hypothesis was that if a collection time constant or thermally induced hemolysis was present in this experiment, some hysteresis should be observable when comparing the upward sweep to the downward sweep. Three downward sweeps were conducted for comparison to previously collected upward sweeps from the same trial (Porcine #3). Given the findings detailed in section 5.3.2, two of the downward sweeps were conducted at low gain settings.

![Up vs Down Sweep in Porcine #3](image)

*Figure 59 – Plot of typical upward sweeps compared with reversed downward sweeps. HG refers to high gain settings; LG refers to low gain settings.*
The results from this particular test are somewhat inconclusive. Given the data in Figure 59, it is possible that there is some uncaptured system time constant. Taken with the temperature data from Figure 60 and the results described in section 5.3.3, it seems more plausible that the discrepancy observed between upward and downward sweeps is a result of thermal influences.

5.3.5 Other Observations

During the course of testing, some additional useful observations were made which might aid understanding of particular aspects of this thesis or otherwise better inform future works. First, a dynamic control was always performed for each trial wherein the rotor was levitating but not spinning. Hemolysis from the dynamic control was always within the bounds of measurement uncertainty of the background samples taken directly
from the bottle. That is, there is no perceptible difference between the dynamic control and background levels. This suggests that contact with the device itself causes no secondary damage to the cells. All the same, it is advisable that this control always be performed at the start of a trial in order to capture any abnormal conditions that may only be present on a given day, such as a residue from cleaning or an incorrectly concentrated saline rinse.

It was also observed that, predictably, background levels of hemolysis consistently increase throughout the day of testing. This is attributed both to non-refrigerated storage during testing and gentle mixing between test charges.

![Background Hemolysis - Porcine Trial #2](image)

*Figure 61 – Chart of background levels of damage in Porcine Trial #2. Pools 2 and 3 were left refrigerated until the preceding pool was half empty, allowing time to reach room temperature before use.*

Due to a shipping error, a large quantity of ovine blood was unexpectedly received which could not be effectively used immediately upon its arrival. In performing some auxiliary experiments two days later, it was discovered that background levels of damage had risen to 0.2%. Given the already low signal of observed ovine blood damage in this work, the resulting data was lost in the noise of the high background. This suggests that when investigating hemolysis on such a sensitive level, it is critical that the time-since-draw be minimal and well controlled between experiments, as it has been in this work. None of the high-background data from that blood pool has been reported as valid in this work.
Typically in this work, plasma samples were read approximately 12 hours after being plated. During this time, they were stored in a refrigerator. However it was observed that this allowed some small amount of evaporated liquid to condense on the lid of the 96-well plate. It is unknown if this affected results, however it seems unlikely as condensation was minimal and all wells seem to condense equally.

In ovine trial #2, a series of random hematocrit checks was performed on collected experiment samples. Minimal variance (less than 1) was found between samples, suggesting that the hematocrit can safely be assumed relatively constant throughout a given test charge and throughout a day’s trial, provided thorough mixing is performed before loading the syringe. That said, in later porcine trials, it was observed that porcine blood separates much more quickly than the ovine blood did, likely owing to the smaller size of the red blood cells. If left to sit for extended periods in the syringe between test charges, it was necessary to mix the blood in the syringe before beginning.

When operating under high heat conditions, it was occasionally observed that there were very fine microbubbles appearing in the fluid at the outlet of the device. This was attributed to the release of dissolved gasses in the blood due to elevated temperatures as there was insufficient heat for boiling to occur. The small percentage of samples in which this occurred were discarded. Under the utmost extreme heat conditions, there was a brown residue discovered in cleaning the device which is likely a result of cooked blood. This was discovered early on in blood testing prior to primary trials and procedures were put in place to ensure device shut-down before it was able to reach these thermal extremes. All presented data is not influenced by these factors.
6 Discussion and Conclusions

6.1 Results in the Context of the Literature

One of the stated goals of this thesis was to make observations which usefully contextualize existing works in the literature. To that end, Figure 62 and Figure 63 depict the primary data collected in this work compared with the power-law surfaces produced by Zhang (2012) and Ding (2015), both from the same research group working with the same device, which is highly similar to the one used in this thesis.

![Ovine Compared to Zhang Power-Law](image)

*Figure 62 – Plot comparing ovine upward sweeps to power-law surface generated from constants provided by Zhang et al. [26].*
These papers assert that ovine blood is considerably more fragile than porcine, however this work observed the opposite. In fact, ovine damage from this work is extremely low comparatively with the surface given by Zhang (2012). One possible explanation of this could be that in that study, blood was taken sacrificially from a slaughterhouse rather than through venipuncture as in this work. Increased fragility in sacrificial slaughterhouse blood has been reported in literature, however Zhang (2012) claims both types were compared and no difference was found [26], [56]. It could also be that the conventional bearings in the device used in Zhang (2012) and Ding (2015) introduced regions of uncharacterized shear which lead to increased levels of observed damage. There is also no mention of fluid temperature or heat contributed by the motor, beyond specifying that the tests were
conducted at room temperature. Any or all of these could be contributing factors to the observed high levels of damage in that work.

Regarding the higher observed damage in the high stress range for porcine blood, this could be a number of factors in either this work or Ding (2015) resulting in an overestimation or underestimation respectively. Given the auxiliary findings discussed in section 5.3 it seems highly likely that in the high shear, high exposure region of the curve, this thesis overestimates shear-induced damage in both species of blood because of some uncaptured thermal or orbit effects. Still present however is the extremely low damage relative to Ding (2015) in the low shear range below 150Pa, which has a high associated confidence and is consistent with findings in Paul et al. (2003) and Boehning et al. (2014) [28], [57].

The findings depicted in section 5.3.2 indicate that low gain settings avoid or reduce whatever secondary damage factors are present at high shear rates within the device. While this is only a small amount of data, given the consistency of the primary results between trials, it seems probable that the collected low gain porcine data is more representative of the true shear-exposure-hemolysis surface in porcine blood. Figure 64 is a reproduction of Figure 63 using only the low gain data. Like the high gain data, though to a lesser extent, the high-shear, long-exposure time data still passes above the Ding (2015) surface. However, it also shows lower damage at low-shear and short-exposure than is seen in the results from Ding (2015), suggesting an overestimation in that work.
6.2 **Strengths of this Experiment**

While this thesis has not produced universally indisputable evidence describing the fragility of red blood cells in rotational medical devices, there are significant merits to this device and this analysis that are not present in comparable works. Given the relatively minimal attention paid to such parameters as blood viscosity, system temperatures, and complex flow structures in their published work, comparable studies were likely affected by similar issues to this work, though it was not captured or acknowledged as it has been here.

A high volume of data has been analyzed in this work with four donor animals per species investigated across ~1500 samples. Primary upward sweep testing revealed a high
level of consistency between trials. Extremely conservative analysis was performed in this work whenever possible. That is, wherever an incompletely characterized parameter exists in this study, it would almost certainly result in a secondary source of additional damage - heat, turbulence, and roughness as examples. Despite this, observed damage has been relatively low, the large majority below 1%, extremely so in the case of ovine blood. This is especially true in comparison to the other similar works in the literature, with the only exception being the high shear, long exposure region for porcine blood. Admittedly, that particular set of conditions results in an almost certain overestimation of fragility. However, hemolysis in the lower ranges of fluid shear is reported with a high level of confidence and is not at all likely influenced by any of the proposed secondary damage factors.

Additionally, the observations regarding hemolysis at different hematocrits and bulk viscosities described in section 5.3.1 are potentially extremely significant to the body of literature at large, pending further, more robust investigation. Hemolysis as a function of stress and exposure time is the contemporary paradigm in the field and these preliminary findings suggest it may be more prudent to examine shear rate, which is not a function of blood viscosity, rather than stress, which is.

6.3 Advised Future Work

The two primary weak points of this work are the thermal effects given by the electrical components and the complex flow structures that are likely present in the system. To that end, it is advisable that any future blood fragility investigation conducted with this device first fully characterizes these effects and remedies them where possible.
First, the incompletely characterized thermal effects are the most obvious limitation of this work. At the time of writing and to the author’s knowledge, there are no significant works in the literature which describe hemolysis induced by brief exposure to a high heat surface, as occurred in this thesis. There have been a number of works that investigate thermally induced hemolysis more generally however. Gershfeld et al. (1988) immersed human blood into baths from 4-50°C over a period of 30 hours and reported minimal variability of hemolysis between 4, 20, 37, 40, and 45°C pools over the first 3 hours [58]. Rakow et al. (1975) observed that the red blood cell membrane elasticity irreversibly and dramatically changes when “heat treated” for several minutes over a narrow temperature range of 45-48.8°C [59]. Baar et al. (1970) heated blood to 50°C for 3 minutes and photographed significant deformation and disruption of the cell’s natural biconcave disk shape [60]. Based on these works, it seems possible that the some portion of the cells in this shearing device are thermally damaged or altered via exposure to the local hot spots of the device under high shear, low flow conditions.

In order to study the thermal effects of this shearing device in more depth, a test cell could be devised wherein the geometry is similar to the current magnetic bearing shearing device but rather than rotate to apply shear, the test cell enables heat to be applied in an equivalent manner and magnitude to what has been observed in this thesis. This would allow the evaluation of hemolysis as a function only of applied heat and residency time within the cell, capturing the degree to which heat directly causes lysis, as well as allow the exposed cells to be examined for evidence of alterations like those observed in the literature. This will not however, reveal if heat causes the cells to become more fragile and thus more susceptible to lysis via applied shear as is suspected in this thesis.
Second, to better quantify the complex flow structures, a test cell could be devised with identical geometry but with the absence of magnetic bearings and brushless motor actuation and instead a conventional bearing shaft with attached rotor, allowing the walls to be made transparent and the internal flow structures to be directly observable via particle image velocimetry (PIV). The critical area of study is the presence of Taylor vortices and turbulence in the shearing gap and in the non-gap region as a function of both rotor speed and axial flowrate. It is also advisable that this experimental approached be supported by a robust CFD analysis of an equivalent geometry.

In the interim of the construction of these test cells, there are some adjustments and parametric studies that could be relatively easily performed with the current magnetic bearing shearing device but were outside the scope of time for this thesis. First, a reliable determination and application of optimal bearing gains could dramatically reduce the heat in the system, possibly removing this as an issue altogether. It may be productive to investigate some dynamic system or control law which adjusts the gains in real time as a function of rotor speed in order to minimize heat as it has been observed that different gains perform better at different speeds. Second, the geometry of the shearing gap region could be easily adjusted and parametrically studied, although it should be noted that any changes will likely affect both the thermal profile of the device as well as any complex flow structures. The primary variables of interest are the ring’s outer diameter (determining the gap size), the gap regions axial length, and the gap’s axial position on the rotor. A larger ring would allow higher shear rates at lower rotor speeds, potentially reducing both heat and flow complexity. An axially longer gap would allow longer exposure times at faster flowrates, reducing residency time in the device and potentially reducing thermal influence.
on hemolysis. Different gap positions may create more ideal rotor dynamics which reduce orbit size and the necessity for bearing power and the associated heat that comes with that. Finally, the shearing ring could be removed entirely or dramatically shrunk. This would effectively remove the part of the device which applies high shear, allowing investigation of the same rotor speed and flow rate ranges as in this thesis and potentially revealing secondary damage sources or characterizing hemolysis due to the thermal effects and complex flows.

If these issues could be sufficiently characterized and resolved, it is advisable that more blood testing be conducted, evaluating hemolysis as a function of applied shear and exposure time in bovine blood as well as human, for better comparison between species as well as to existing literature. Further, shear-induced platelet activation is a well known problem in rotational medical devices which can lead to thromboses and bleeding – both severe complications for a patient. Like hemolysis, this area is not wholly understood and quantified. It would be productive to adapt this device and experiment procedure to an investigation in that related field.

This thesis only explored the exposure of cells to a single pass-through of high shear flow when, in fact, an implanted VAD would cause blood to recirculate through the high shear regions of the pump repeatedly as it flows through the body. Assuming a round-trip-time of approximately 1 minute, any given red blood cell may experience these shears thousands of times in its life cycle as it circulates through the body. To that end, it would be useful to explore hemolysis over repeated passes through a controlled shear flow as in this
device. This could be easily accomplished using this shearing device with only minor modifications to the apparatus and test methodology.

It is also strongly recommended that further study of the significance of bulk viscosity and plasma viscosity to shear-induced hemolysis, and perhaps other forms of blood damage, be conducted. More specifically, an experiment investigating whether applied stress or applied shear rate is the more apt parameter for discussion in the community and as a device design criteria. The observations made in this thesis suggest that a variable hematocrit, and by extension bulk viscosity, has little effect on resulting damage at a shear-rate. However, the plasma viscosity in these experiments can be assumed constant, and this may be the more relevant parameter to the experienced stress of the cell. Any future work in this area should specifically seek to evaluate the significance of plasma viscosity to experienced damage as well as the bulk viscosity. This may be further supported by an analytical model of the mechanical strain experienced by a single, plasma-suspended cell as a result of applied fluid shear, perhaps making considerations for the different modes of motion a cell can experience.
Appendices

**Digital Appendix:**

For access to the digital repository of all documents pertaining to the completion of this thesis contact Dr. Steven Day - swdeme@rit.edu.

The digital appendix includes, but is not limited to:

- Raw data files
- Data processing files (MATLAB and Excel)
- High-resolution figures
- Video footage of primary blood testing
- Presentation materials

**Appendix A: Reproduced Results in Terms of Shear Rate**

![Observed Hemolysis in Ovine Trials](image)

*Figure 65- Reproduction of Figure 38 in terms of shear rate.*
Figure 66 – Reproduction of Figure 40 in terms of shear rate.

Figure 67 - Reproduction of Figure 41 in terms of shear rate.
Appendices

Figure 68 - Reproduction of Figure 42 in terms of shear rate.

Figure 69 - Reproduction of Figure 43 in terms of shear rate.
Figure 70 - Reproduction of Figure 44 in terms of shear rate.

Figure 71 - Reproduction of Figure 45 in terms of shear rate.
Figure 72 - Reproduction of Figure 46 in terms of shear rate.

Figure 73 - Reproduction of Figure 58 in terms of shear rate.
Figure 74 - Reproduction of Figure 59 in terms of shear rate.

Figure 75 - Reproduction of Figure 60 in terms of shear rate.
Appendix B: MATLAB Data Analysis Script

%LVAD Data Report Generation
%James Krisher, Master's Thesis Aug. 2018
clc; clear;

filepath = 'F:\RIT\Research\Test Results\8-2 Porcine Blood';
% Check drive***

Afilename = {
    '08-02-18_A.1.lvm';
    '08-02-18_B.1.lvm';
    '08-02-18_C.1.lvm';
    '08-02-18_D.1.lvm';
    '08-02-18_E.1.lvm';
    '08-02-18_F.1.lvm';
    '08-02-18_G.1.lvm';
    '08-02-18_H.1.lvm';
    '08-02-18_I.1.lvm';
    '08-02-18_J.1.lvm';
    '08-02-18_K.1.lvm';
    '08-02-18_L.1.lvm';
    '08-02-18_M.1.lvm';
    '08-02-18_N.1.lvm';
    '08-02-18_O.1.lvm';
    '08-02-18_P.1.lvm';
};

Anomflow = [
    9;
    5.5;
    9;
    11.5;
    15.8;
    25.2;
    9;
    5.5;
    9;
    25.2;
    5.5;
    25.2;
    9;
    11.5;
    25.2;
    9;
];
%correction = 1.055762;
correction = 1;

for r = 1:length(Afilename)
    close all
    filename = char(Afilename(r)) % ex.'02-13-18_A.1.lvm'
    testindex= filename(10);

    nomflowrate = Anomflow(r) %ml/mn *** enter nominal flowrate of charge.
This will be modified by $y = 1.0237x + 0.0942$ to account for syringe error.

flowrate = nomflowrate*1.075;  % Old Syringe Pump
flowrate = nomflowrate*1.0237 + 0.0942;  % ml/mn  % Harvard pH

volumeconst1 = 7.5;  % ml - device plus tubing
volumeconst2 = 6;  % ml - device
timeoffset1 = (volumeconst1/flowrate)*60;
timeoffset2 = (volumeconst2/flowrate)*60;

threshold = 0.075;
thresholdS = threshold*3;

% Import Data File
effpath = strcat(filepath, filename);
rawdat = lvm_import(effpath);
tempdiff = rawdat.Segment1.data(:,13) - rawdat.Segment1.data(:,12);
rawdatindex = 1:length(rawdat.Segment1.data(:,1));

% Parse Out Samples
i=1;
n=1;
sample = zeros(1,2);
for i=2:length(rawdat.Segment1.data(:,1)) - 1
    alpha = rawdat.Segment1.data(i,14);
    beta = rawdat.Segment1.data(i-1,14);
    gamma = rawdat.Segment1.data(i+1,14);
    if alpha-beta == 1
        sample(n,1) = i;
    end
    if gamma-alpha == -1
        sample(n,2) = i;
        n = n+1;
    end
end

sample

samplenumber = size(sample,1)
i=1;
sampletime = zeros(size(sample,1),1);
samplelength = zeros(size(sample,1),1);
samplevol = zeros(size(sample,1),1);
for i=1:size(sample,1)
    sampletime(i) = rawdat.Segment1.data(sample(i,2),1) - rawdat.Segment1.data(sample(i,1),1);
    samplelength(i) = sample(i,2) - sample(i,1);
    samplevol(i) = sampletime(i)*flowrate/60;
end
sampletime

samplelength

samplevol

filtstress = medfilt1(correction*rawdat.Segment1.data(:,7),40);
i=1;
Tsample = zeros(size(sample,1),26);
ptchid = logical(zeros(size(sample,1),length(rawdatindex)));
boxdat = [];
boxid = [];


Appendices
for i=1:size(sample,1)
    recindex=sample(i,1);
    rect = rawdat.Segment1.data(recindex,1);
    INt = rect-timeoffset1;
    OUTt = INt + sampletime(i)+timeoffset2;
    possiblesIN = abs(rawdat.Segment1.data(:,1)-INt)<threshold;
    if possiblesIN == 0
        possiblesIN = abs(rawdat.Segment1.data(:,1)-INt)<thresholdS;
    end
    indexIN = find(possiblesIN); % possible indices
    a= min(indexIN);
    if isempty(a)
        a = 1;
        disp('PURGE TIME WAS INSUFFICIENT')
    end
    possiblesOUT = abs(rawdat.Segment1.data(:,1)-OUTt)<threshold;
    if possiblesOUT ==0
        possiblesOUT = abs(rawdat.Segment1.data(:,1)-OUTt)<thresholdS;
    end
    indexOUT = find(possiblesOUT); % possible indices
    b= max(indexOUT);
    Tsample(i,1) = i;
    Tsample(i,2) = sampletime(i); % Duration
    Tsample(i,3) = mean(rawdat.Segment1.data(a:b,3));
    Tsample(i,4) = std(rawdat.Segment1.data(a:b,3));
    Tsample(i,5) = mean(correction*rawdat.Segment1.data(a:b,4)); % Shear Rate
    Tsample(i,6) = std(correction*rawdat.Segment1.data(a:b,4)); % Shear Rate
    Tsample(i,7) = mean(rawdat.Segment1.data(a:b,6));
    Tsample(i,8) = std(rawdat.Segment1.data(a:b,6));
    Tsample(i,9) = mean(correction*rawdat.Segment1.data(a:b,7)); % Stress
    Tsample(i,10) = std(correction*rawdat.Segment1.data(a:b,7)); % Stress
    Tsample(i,11) = max(filtstress(a:b))-Tsample(i,9);
    Tsample(i,12) = Tsample(i,9)-min(filtstress(a:b));
    Tsample(i,13) = mean(rawdat.Segment1.data(a:b,8));
    Tsample(i,14) = std(rawdat.Segment1.data(a:b,8));
    Tsample(i,15) = mean(rawdat.Segment1.data(a:b,9));
    Tsample(i,16) = std(rawdat.Segment1.data(a:b,9));
    Tsample(i,17) = mean(rawdat.Segment1.data(a:b,10));
    Tsample(i,18) = std(rawdat.Segment1.data(a:b,10));
    Tsample(i,19) = mean(rawdat.Segment1.data(a:b,11));
    Tsample(i,20) = std(rawdat.Segment1.data(a:b,11));
    Tsample(i,21) = mean(rawdat.Segment1.data(a:b,12));
    Tsample(i,22) = std(rawdat.Segment1.data(a:b,12));
    Tsample(i,23) = mean(rawdat.Segment1.data(a:b,13));
    Tsample(i,24) = std(rawdat.Segment1.data(a:b,13));
    Tsample(i,25) = mean(tempdiff(a:b));
    Tsample(i,26) = std(tempdiff(a:b));

    ptchid(i,a:b) = 1; % Assign window for shading later
    C(1:length(a:b),1) = i;
    boxdat = [boxdat ; correction*rawdat.Segment1.data(a:b,7)];
    boxid = [boxid ; C];
    C = [];
end

%% Plot Generation
figure(1) % Time Dependent Device Features
clf;
subplot(6,1,1) % Voltage
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,2))
title(strcat(filename(1:end-4), ' Time Vs Device Properties'), ... 'Interpreter', 'none')
ylabel(rawdat.Segment1.column_labels{2})
ylim([0 10])
grid on
subplot(6,1,2) % Rotor Speed
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,3))
ylabel(rawdat.Segment1.column_labels{3})
ylim([0 15000])
grid on
subplot(6,1,3) % Current
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,5))
ylabel(rawdat.Segment1.column_labels{5})
ylim([0 3])
grid on
subplot(6,1,4) % Orbits
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,8), 'c', ... rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,9), 'm', ... rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,10), 'y')
ylabel('Orbit Std. Dev. [um]')
ylim([0 200])
legend('F', 'R', 'I', 'Location', 'northwest')
grid on
subplot(6,1,5) % Pressure
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,11))
ylabel(rawdat.Segment1.column_labels{11})
ylim([0 80])
grid on
subplot(6,1,6) % Temperature
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,12), ... rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,13))
xlabel('Time [s]')
ylabel('Temperature [C]')
ylim([10 50])
legend('T1', 'T2', 'Location', 'northwest')
grid on
annotation('textbox',[0 0 .2 .05], ... 'String',rawdat.Segment1.column_labels{20}, ... 'FitBoxToText','on')

figure(2) % Time Dependent Fluid Features
clf;
subplot(6,1,1) % Rotor Speed
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,3))
title(strcat(filename(1:end-4), ' Time Vs Fluid Properties'), ... 'Interpreter', 'none')
ylabel(rawdat.Segment1.column_labels{3})
ylim([0 15000])
grid on
subplot(6,1,2) % Viscosity
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,6))
ylabel(rawdat.Segment1.column_labels{6})
ylim([0 7])
grid on
subplot(6,1,3) %Stress
plot(rawdat.Segment1.data(:,1),correction*rawdat.Segment1.data(:,7))
ylabel(rawdat.Segment1.column_labels{7})
ylim([0 350])
grid on
subplot(6,1,4) %Orbits
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,8),'c', ...
     rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,9),'m', ...
     rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,10),'y')
ylabel('Orbit Std. Dev. [um]')
ylim([0 200])
legend('F','R','I','Location','northwest')
grid on
subplot(6,1,5) %Pressure
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,11))
ylabel(rawdat.Segment1.column_labels{11})
ylim([0 80])
grid on
subplot(6,1,6) %Temperature
plot(rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,12), ...
     rawdat.Segment1.data(:,1),rawdat.Segment1.data(:,13))
xlabel('Time [s]')
ylabel('Temperature [C]')
ylim([10 50])
legend('T1','T2','Location','northwest')
grid on
annotation('textbox',[0 0 .2 .05], ...
     'String',rawdat.Segment1.column_labels{20}, ...
     'FitBoxToText','on')

figure(3) %Voltage Dependent
cif;
subplot(3,1,1) %Rotor Speed
plot(rawdat.Segment1.data(:,2),rawdat.Segment1.data(:,3),'.'
     title(strcat(filename(1:end-4), ' Voltage Dependent Plots'), ...
     'Interpreter', 'none')
ylabel(rawdat.Segment1.column_labels{3})
ylim([0 15000])
grid on
subplot(3,1,2) %Current
plot(rawdat.Segment1.data(:,2),rawdat.Segment1.data(:,5),'.'
     ylabel(rawdat.Segment1.column_labels{5})
ylim([0 3])
grid on
subplot(3,1,3) %Orbit
plot(rawdat.Segment1.data(:,2),rawdat.Segment1.data(:,8),'c.', ...
     rawdat.Segment1.data(:,2),rawdat.Segment1.data(:,9),'m.', ...
     rawdat.Segment1.data(:,2),rawdat.Segment1.data(:,10),'y.')
xlabel('Voltage [V]')
ylabel('Orbit Std. Dev. [um]')
ylim([0 200])
legend('F','R','I','Location','northwest')
grid on
annotation('textbox',[0 0 .2 .05], ...
figure(4) % Speed Dependent
clf;
subplot(5,1,1) % Current
plot(rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,5),'.')
title(strcat(filename(1:end-4), ' Speed Dependent Plots'), ...
'Interpreter', 'none')
ylabel(rawdat.Segment1.column_labels{5})
ylim([0 3])
grid on
subplot(5,1,2) % Viscosity
plot(rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,6),'.')
ylabel(rawdat.Segment1.column_labels{6})
ylim([0 7])
grid on
subplot(5,1,3) % Stress
plot(rawdat.Segment1.data(:,3),correction*rawdat.Segment1.data(:,7),'.')
ylabel(rawdat.Segment1.column_labels{7})
ylim([0 350])
grid on
subplot(5,1,4) % Orbit
plot(rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,8),'c.', ...
rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,9),'m.', ...
rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,10),'y')
ylabel('Orbit Std. Dev. [um]')
ylim([0 200])
legend('F','R','I','Location','northwest')
grid on
subplot(5,1,5) % Temperature
plot(rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,12),'.', ...
rawdat.Segment1.data(:,3),rawdat.Segment1.data(:,13),'.')
xlabel('Speed [RPM]')
ylabel('Temperature [C]')
ylim([10 50])
legend('T1','T2','Location','northwest')
grid on
annotation('textbox',[0 .2 .05], ...
'String',rawdat.Segment1.column_labels{20}, ...
'FitBoxToText','on')

% Sample Distribution
t=rawdat.Segment1.data(:,1);
yl=correction*rawdat.Segment1.data(:,7);
strsamplenum = num2str(samplenum);
figure(5)
clf;
h = plot(rawdat.Segment1.data(:,1),correction*rawdat.Segment1.data(:,7), ...
rawdat.Segment1.data(:,1),filtstress,'g');
set(h(1),'LineWidth',1)
set(h(2),'LineWidth',2)
ylabel(rawdat.Segment1.column_labels{7})
ylim([0 350])
xlabel('Time [s]')
title(strcat(filename(1:end-4), 'Sample Distribution'),...
'Interpreter', 'none')
grid on
for i=1:size(sample,1)
    if (i==floor(size(sample,1)/2) || i==1 || i==size(sample,1))
        patch([t(ptchid(i,:)); flipud(t(ptchid(i,:)))], [y1(ptchid(i,:)); zeros(size(y1(ptchid(i,:))))]), [0.6 0.4 0.9], 'FaceAlpha', 0.3,
        'EdgeColor', 'r')
    end
    patch([t(ptchid(i,:)); flipud(t(ptchid(i,:)))], [y1(ptchid(i,:)); zeros(size(y1(ptchid(i,:))))]), [0.6 0.4 0.9], 'FaceAlpha', 0.3,
        'EdgeColor', 'none')
end
annotation('textbox',[0 0 .2 .05],...
'String',strcat(rawdat.Segment1.column_labels{20},',',strsamplenum, ', samples taken. First, middle, and final are highlighted.'),...
'FitBoxToText','on')

figure(6)
clf;
boxplot(boxdat,boxid)
ylabel(strcat(rawdat.Segment1.column_labels{7}))
ylim([0 350])
xlabel('Sample Number')
title(strcat(filename(1:end-4), 'Sample Box and Wiskers'),...
'Interpreter', 'none')
grid on

%% Saving
cd(filepath)
set(figure(1),'PaperUnits','inches','PaperPosition',[0 0 10 10])
saveas(figure(1),fignam);
saveas(figure(1),fignam)
set(figure(2),'PaperUnits','inches','PaperPosition',[0 0 10 10])
fignam = strcat(filename(1:end-4),'_F2TimeVsFluidProp.png');
saveas(figure(2),fignam)
set(figure(3),'PaperUnits','inches','PaperPosition',[0 0 10 10])
fignam = strcat(filename(1:end-4),'_F3VoltageDep.png');
saveas(figure(3),fignam)
set(figure(4),'PaperUnits','inches','PaperPosition',[0 0 10 10])
fignam = strcat(filename(1:end-4),'_F4SpeedDep.png');
saveas(figure(4),fignam)
set(figure(5),'PaperUnits','inches','PaperPosition',[0 0 15 10])
fignam = strcat(filename(1:end-4),'_F5SampDist.png');
saveas(figure(5),fignam)
set(figure(6),'PaperUnits','inches','PaperPosition',[0 0 15 10])
fignam = strcat(filename(1:end-4),'_F6BoxWisk.png');
saveas(figure(6),fignam)
xlnam = strcat(filename(1:9),'sampledata.xlsx');
xlswrite(xlnam,Tsample,filename(1:end-4))
end
References


[26] T. Zhang et al., “Study of flow-induced hemolysis using novel couette-type blood-


