Experimental Estimation of Human Blood Plasma Viscosity

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***Abstract*—Plasma viscosity is an important parameter in biology and fluid mechanic. This parameter is linked to several diseases in human. Usually to measure the plasma viscosity, red blood cells should be separated from a whole blood. This process is time consuming and needs expensive viscometers. In this paper an innovative method is suggested to measure blood viscosity which does not require separating the red blood cells from plasma and it is done in a very small amount time. In this method blood viscosity is measured by finding red blood cells terminal velocity in sedimentation. An experimental set-up is designed using transparent polydimethylsiloxane (PDMS) material and video microscopic system. Image processing techniques are used to detect red blood cells and their respected velocity. Using this velocity, plasma viscosity can be estimated using force balance on red blood cells. The viscosity measured by this algorithm then is compared with the results of a viscometer.**

***Index Terms*—plasma viscosity, red blood cells, image processing, red blood cells sedimentation**

I. Introduction

Plasma viscosity is linked to many diseases, several studies were conducted on the relationship of plasma viscosity with malfunction in the human body system. Caerphilly and Speedwell studies, also the MONICA project suggested that plasma viscosity is a predictive risk factor for Coronary heart disease [1], [2]. For patients with unstable angina pectoris, high plasma viscosity can also lead to an increased risk of acute myocardial infarction [3], [4]. Moreover, several studies on myocardial infarction indicated a relationship of an early increase in plasma viscosity during acute myocardial infarction and reinfarction or death [5], [6]. Evidently, plasma viscosity contributes to the cardiovascular risk and also has an important role in reducing blood flow, which mostly occurs in patients with advanced atherosclerosis [7]-[11]. For a normal human body system, the plasma viscosity range is unrelated to age and gender and narrow between 1.10-1.30 mPa.s at 37°C, only little changes are pathologically important [12]-[14]. Despite the fact that plasma viscosity is not influenced by red blood cell aggregation, hematocrit (anemia, polycythemia) or hemoglobinopathias, it can increase in parallel with erythrocyte sedimentation rate (ESR). Because of these approving features, plasma viscosity test was proposed to replace ESR [12- 14] in 1942.

Plasma is a Newtonian fluid; its viscosity is independent of blood flow characteristics and it is determined basically by water content and macromolecular components of blood [15]. Many techniques and procedures are used for measuring the fluid viscosity. Methods are ranging from measuring the time of flowing fluids through capillaries to fluid searing viscometers (shear the fluid between rotating cup and bob or cone and plate type assemblies) [16] ,[17]. The minimum fluid sample volume needed to measure the fluid viscosity in viscosity measuring techniques is important. Several types of viscometers are used to measure the viscosity of plasma. For each specific method, the required liquid sample volume varies from a few hundred microliters to milliliters [18], [19].

In this research whole blood is used as the sample in the sedimentation test. Using whole blood, results in having the aggregation phenomena in the domain. Aggregation of red blood cells is a reversible stacking of red blood cells in stagnated plasma. The stacks particles settle faster than dispersed single red blood cells. Due to close value of plasma density and red blood cells density, giving time, only dispersed single red blood cells remain on top of the domain in the sedimentation test. These single red blood cells reach to terminal settling velocity in a very short amount of time. To develop this viscosity measurement method, image processing techniques are used extensively to find and measure the terminal velocity. Using balance of force and Stokes equation, a relation between terminal velocity and viscosity of plasma is derived. The viscosity measured by this algorithm then is compared with results of a viscometer (Rheosense INC, San Ramon, CA, USA).

In the next section, the developed method of viscosity measurement for plasma is explained. This method needs only 0.5 ml of whole blood sample instead of separated

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plasma. It requires a minimum amount of time, approximately 10 minutes, therefore, it can be used as a point of care device to measure plasma viscosity.

II. Proposed Methods and Procedures

A. Background Theory

Particles settling in a liquid, reach terminal velocity after a few moments depending on the viscosity of the liquid. There is a force balance between drag and gravity forces when particles get to the terminal velocity. In this research, this balance of forces is subjected to study. The viscosity has a direct effect on the drag force and terminal velocity. By measuring these parameters in the experiment one can find the viscosity of plasma.

The gravity force on a single red blood cell settling can be presented as

$$F_g = \left(\rho_{RBC} - \rho_{Plasma}\right) g V_{RBC} \tag{1}$$

Where g is the gravitational acceleration, ρ_{RBC} is the density of the particles and equal to 1125 kg/m^3. The mass density of the plasma, ρ_{plasma}, is 1025 kg/m^3. Volume of single red blood cell, V_{RBC} is assumed to be 90 micrometer^3. [20]

The drag force on a red blood cells can be calculated as

$$F_D = \frac{1}{2} C_D \rho v^2 A \tag{2}$$

Typical Reynolds number in this study is in order of 10^{-4}. Where v is the flow velocity relative to the object (in this study, it is the terminal velocity of the falling red blood cells), C_D is the drag coefficient, v is the particle velocity and A is the cross sectional area.

Stokes derived the following equation for drag coefficient of low Reynolds number spherical particles.

$$C_D = \frac{24}{\mathrm{Re}} \tag{3}$$

Where:

$$\mathrm{Re} = \frac{\rho v d}{\mu} \tag{4}$$

Now by combining Equations (2), (3) and (4), the following equation can be derived

$$F_D = 6\pi\mu R v \tag{5}$$

Where μ is the dynamic viscosity, R is the radius of the spherical object.

The main assumption for Stokes low comes from the fact that it is driven for the spherical particles. This assumption is critical for this study and it is taken in to the consideration when comparing viscometer's results to the results generated by presented algorithm.

By invoking Newton first law and balance of forces shown in Equation (1) and (5), viscosity can be found by

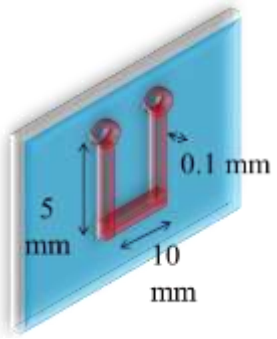
$$\mu = \frac{\left(\rho_g - \rho_f\right) g V_{RBC}}{6\pi R v} \tag{6}$$

B. Micro-Channel Fabrication

In order to perform the experimental test, a proper channel should be designed and fabricated. First, Poly-Di-Methyl-Siloxane (PDMS) is used for the fabrication of channels. PDMS is formed from a mixture of silicon base elastomer and a curing agent at a ratio of 10 to 1 to obtain the appropriate stiffness suitable for the application.

The method used to prepare the channels starts by designing a mask using "AutoCAD" software. Completed designs are sent to special commercial printers for accurate printing. Once the mask is completed, the mold needs to be created. For this step, negative photoresist is spin coated on a silicon wafer and exposed to UV lights. A complete protocol for this procedure has been provided by Renaud *et al.* [21].

After preparation of the mold, the PDMS is poured on the glass slide, then it is heated for 90 minutes at 80oC to obtain a flexible but solid structure that is cut to separate the single channels. The channels are then punched at the channel inlets to insert the connectors of 2.5 mm diameter. Finally the punched channels are bonded to a microscope glass slide. The bonding process utilizes the oxygen plasma bonding method using the PE-50 series plasma system (Plasma Etch, USA). When the two surfaces to be bonded are exposed to the oxygen plasma, the surface layers with the lower molecular weight are removed while the uppermost atomic layer of the polymer is oxidized. The advantage of using this method is the non-reversible bonding created that comes with clean surfaces to avoid blockage of the channel. The treated surfaces engender a hydrophilic behavior of the PDMS [22] that lasts for at least 150 hours [23].



a) U shaped set-up

b) Measuring the height of channel

Figure 1. Sedimentation test set-up and dimension.

For sedimentation study, a U shape channel with 100 μm height is used [24]. The different lengths of this channel can be viewed in Fig. 1(a). Measurement of the height is done by cutting the channel and looking at it under the microscope (Fig. 1(b)).

C. Experiment Procedure

Six Healthy human whole blood samples are used to evaluate the viscosity of plasma. The blood is collected into a tube containing EDTA.

Experimental set-up shown in Fig. 2 is used for this study. It consists of a high speed camera (Graftek Imaging, Inc., Austin, TX, USA) controlled using LabVIEW software (National Instruments, USA), vertical microscope (Axio Lab. A1) with 10x lens magnification and a white light source.

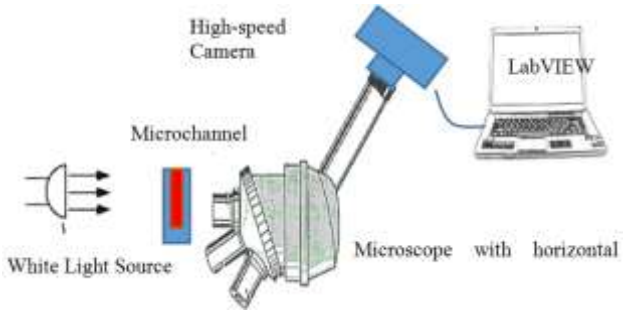


Figure 2. Video microscopic system used in the sedimentation experiment.

Images are recorded at the rate of one frame per second. The frames capture is controlled via a program developed in LabVIEW, where parameters such as the exposure time of the camera, the frame rate and the field of view can be varied to obtain the highest image quality for proper post processing. A sample of the images at the beginning of the experiments is shown in Fig. 3.

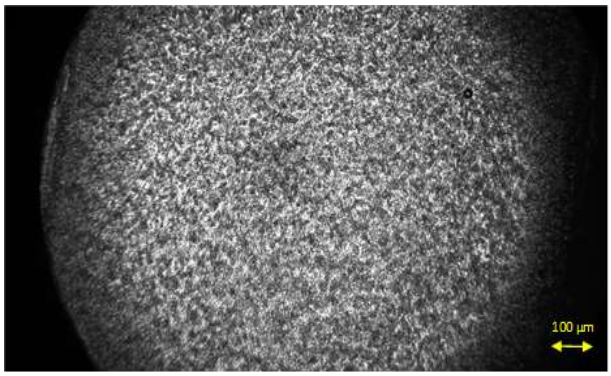


Figure 3. Recorded image using 10x lens magnification and high-speed camera.

Red blood cells start to aggregate and form bigger particles. These particles settle faster due to less drag force applied on them. After about 360 seconds, only dispersed single red blood cells remain in the field of view. Due to scattered distribution of single red blood cells, the possibility of a collusion and aggregation reduces (Fig. 4).

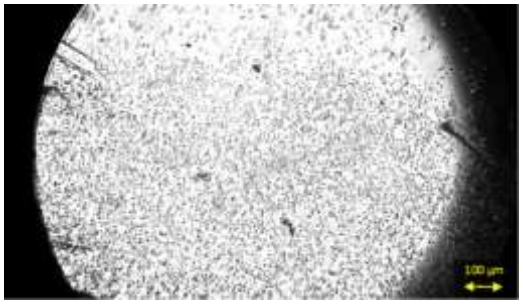
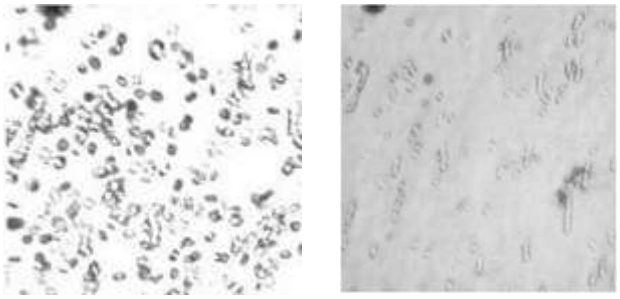


Figure 4. Recorded image after 360 seconds.

D. Image Processing

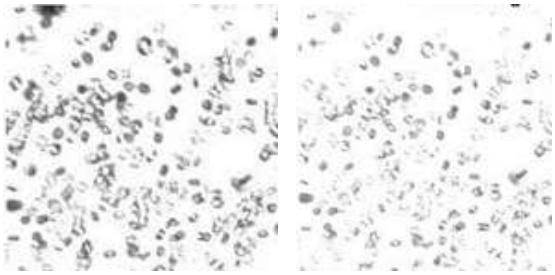
To reduce effects of dust particles on the optic lens and also red blood cells that are attached to the transparent wall, the background image is subtracted from the original image. Removing background reduces the false detection of particles and increases the accuracy of the image processing method. To generate the background, the average of all experimental images is calculated (Fig. 5 错误!未找到引用源。).



a) main image b) background image

Figure 5. Picture taken using video-microscopy system and background image generated by averaging of all the pictures.

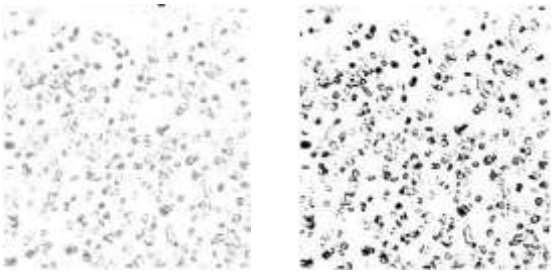
By removing the background image, an image containing only the moving red blood cells is generated. Subtracted image generated from this procedure is shown in Fig. 6. Removing background removes the noises and statics objects.



a) original image b) Subtracted image

Figure 6. Comparison of the original image with background subtracted image.

The next step in the image processing is to change grey scale image to a binary image. This procedure is called thresholding. To make it easier for thresholding step and tracking the moving red blood cells, the contrast of images is increased by considering the contrast limited-adaptive histogram equalization (Fig. 7). This way removes the sensitivity of thresholding criteria.



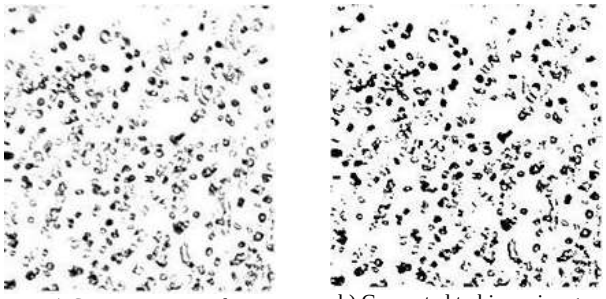
a) Subtracted image b) Contrast increased

Figure 7. Applying contrast limited adoptive histogram equalization.

The resulted images then is converted into a binary image (black and white) I_{binary} (x; y) according to a set threshold, T, as follows:

$$I_{binary}(x,y)=\begin{cases}1 & if \quad I_{sustracted}(x,y)>T\\ 0 & if \quad I_{sustracted}(x,y)\le T\end{cases} \quad (7)$$

Subtracted image after converting to binary version is shown in Fig. 8. Using the contrast limited-adoptive histogram equalization results in a constant threshold, T, equal to 0.7.



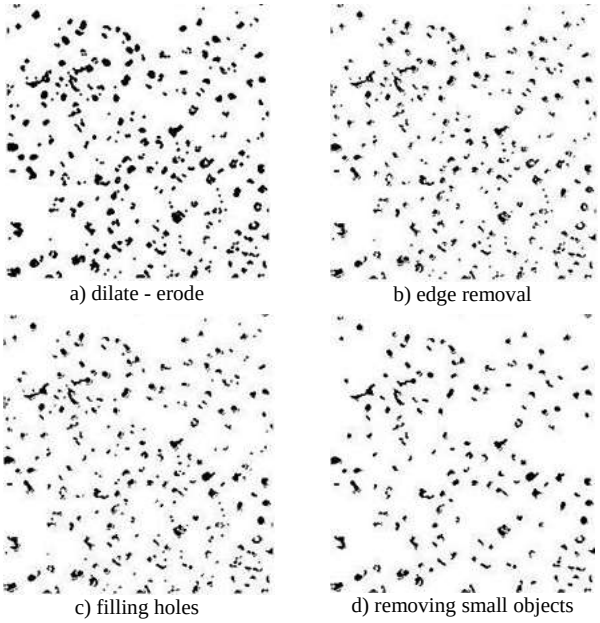
a) Contrast increased b) Converted to binary image

Figure 8. Applying threshold to change a grey scale image to a binary image.

To isolate red blood cells in the images following binary operation are done after thresholding: 1- Dilate, 2- Erode, 3- remove edges 4- Fill holes, 5- Removing small objects. The effects of each binary operator are shown in Fig. 9.

E. Velocity Measurement

The method which is used to find the particle velocity distribution tracks each individual particle and calculates their velocities. This method has a disadvantage that should be considered. For example, the apparent size or center of projected area might change suddenly due to overlapping particles. To face this problem, sudden changes are disregarded and if a particle is not found in the next frame, it is considered as disappeared particle. In this scenario, the history of particle is saved until the time of disappearance. The base code for this tracking method is available in [25]. This code is modified to get its input from the image-processing code. To track particles, particles in each image are linked to the same particle in next image as they move along time. For frame to frame linking of particles the Hungarian algorithm (Munkres algorithm) [26] is used. In this method particle are linked according to their previous velocity and the guessed new location. The code looks for the nearest neighbor to the guessed location using Euclidean distance. If it cannot find a neighbor, that particle is not tracked anymore and no velocity is reported for it. Using this method on experiment's images, velocity distribution of red blood cells is found.



a) dilate - erode b) edge removal

c) filling holes d) removing small objects

Figure 9. Binary operators applied.

F. Direct Plasma Viscosity Measurement

To compare results of this method to actual viscosity, whole blood is centrifuged and plasma is separated. A sample of plasma is used in the viscometer (Rheosense INC, San Ramon, CA, USA) to measure the viscosity of plasma. All the experiments are conducted in the room temperature and this procedure is conducted for all six samples.

III. Results

Using image processing and particle tracking method explained, the velocity distribution of red blood cells in the experiment can be found (shown in Fig. 10). This measurement is done between 360 to 480 seconds of experiment.

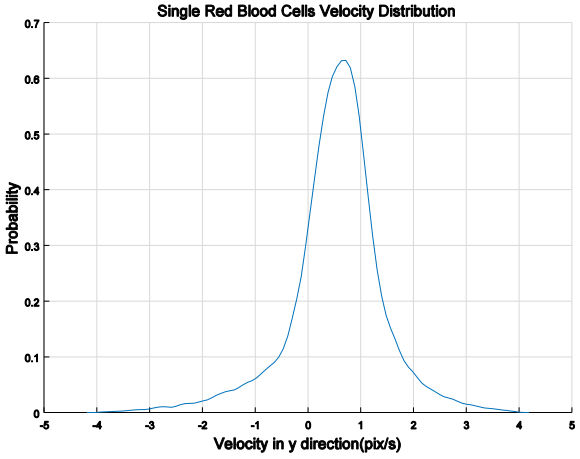


Figure 10. Single red blood cell velocity distribution.

As it can be seen in this graph the probability of single red blood cell having a velocity of 0.72 pixel/second is close to 0.63%. Pixel to micrometer conversion factor is 0.86. This results in a velocity of 0.69 micrometer/second. Using Equation (6) the viscosity can be approximated as 1.1 mPa.s. Similarly for other five samples the results are shown in Table I.

The viscosity found using the viscometer (Rheosense INC, San Ramon, CA, USA) shows plasma viscosity of 1.17 mPa.s for the same sample. Similarly for other samples the results are shown in Table I. This difference of result is coming from the main assumption that is made in this research. It is assumed that the red blood cells are spherical particles and same drag force as spherical particles are applied on them. In the next section, the effect of this assumption is discussed further.

TABLE I. ACTUAL VISCOSITY AND CALCULATED VISCOSITY OF SAMPLES

Sample number	Actual viscosity (mPa.s)	Measured velocity (pixel/s)	Calculated viscosity (mPa.s)
1	1.17	0.72	1.1
2	1.23	0.68	1.13
3	1.13	0.75	1.06
4	1.19	0.72	1.11
5	1.06	0.77	1.01
6	1.27	0.65	1.19

IV. DISCUSSION AND CONCLUSION

Experiments are designed such that the characteristics of plasma viscosity can be found from the terminal velocity of single red blood cells. These experiments are analyzed using image processing techniques to find single red blood cell velocity distribution. The most probable velocity of particles is their terminal velocity. Using this velocity, measured from experiment and balance of forces driven from stokes drag equation, viscosity can be calculated.

Six healthy human blood samples are used for the experiment. To compare, blood plasma is separated from whole blood and its viscosity is measured by viscometer. The results of both measuring methods show agreeable results.

The main assumption made in this research is that red blood cells behave similar to spherical particles. This simplification will lead to error when calculating the viscosity. However this simplification would result in a viscosity measuring system that does not need plasma separation. Other benefits of this system are small amount of blood required and fast process for measuring.

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