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Experimental Correlation for Erythrocyte Aggregation Rate in Population Balance Modeling

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***Abstract*—**Red Blood Cells (RBCs) or erythrocytes tend to form chain-like aggregates under low shear rate called rouleaux. This is a reversible process and rouleaux disaggregate in high shear rates. Therefore, RBCs aggregation occurs in the microcirculation where low shear rates are present but does not occur under normal physiological conditions in large arteries. Numerical modeling of RBCs interactions is fundamental in analytical models of a blood flow in microcirculation. Population Balance Modeling (PBM) is particularly useful for studying problems where particles agglomerate and break in a two phase flow systems to find flow characteristics. In this method, the elementary particles lose their individual identity due to continuous destructions and recreations by break-up and agglomeration. The aim of this study is to find RBCs aggregation in a dynamic situation. Simplified PBM was used previously to find the aggregation rate on a static observation of the RBCs aggregation in a drop of blood under the microscope. To find aggregation rate in a dynamic situation we propose an experimental set up testing RBCs sedimentation. In this test, RBCs interact and aggregate to form rouleaux. In this configuration, disaggregation can be neglected due to low shear stress. A high-speed camera is used to acquire video-microscopic pictures of the process. The sizes of the aggregates and velocity of sedimentation are extracted using an image processing techniques. Based on the data collection from 5 healthy human blood samples, the aggregation rate was estimated as $2.7x10^3(\pm 0.3\ x10^3)$ $1/s$.

***Keywords*—**Red blood cell, Rouleaux, microfluidics, image processing, population balance modeling.

I. Introduction

BLOOD has a unique set of properties as a complex fluid. Blood shows non-Newtonian characteristics in microcirculation where low shear rates (lower than $100s^{-1}$) are present. However, blood behaves differently under normal physiological conditions in large arteries where high shear rates are present [1]. This behavior is related to the particular nature of the blood component; as it is composed of a Newtonian base fluid (plasma) with suspended particles (RBCs, white blood cells, platelets, etc.). RBCs are the most abundant cell in blood and hence are responsible of the changes in blood behavior. However white blood cells, platelets and other component of blood have a negligible effect on blood rheology [2].

RBCs tend to clump together and form regular stacks called rouleaux. This phenomenon is defined as red blood cells aggregation. These stacks are not static, and constantly move and break apart. This is a healthy part of the blood function, and can be viewed as a natural function preserving a more constant set of properties in the human body [3]. This phenomenon was first observed by Fåhraeus in 1921 and described in [4], where the relationship between the aggregate formation and the sedimentation speed was investigated. From his research, the structure of aggregates in healthy and non-healthy individuals was clearly differentiated [5]. There is evidence that aggregation of red blood cells plays a crucial role in regulating blood viscosity [6]. Bureau et al. [7] showed that blood has both thixotropic and viscoelastic behavior which is mainly due to RBCs aggregation. Recently, Owens [8] proposed a model for blood viscosity as a function of rouleaux size. His model is based on Population Balance Equation (PBE) to find rouleaux size. As mentioned in [8], due to lack of information on aggregation and disaggregation rates, a linear relation between disaggregation rate and shear rate was assumed.

Population balance modeling is a practical two phase flow model where particles agglomerate and break are modeled to find flow characteristics. The population balance equation is based on an experimental expression as it ignores microscopic interactions. Therefore, in order to use this equation, it is crucial to develop these experimental expressions.

In this paper, video microscopic is used to obtain sequential images of RBCs and rouleaux. Rouleaux sizes are estimated using image processing techniques be used in numerical simulations. Shiga et al. used particle analyzer software with a television camera to capture either human or rat RBC aggregate formation at different shear rates within a transparent cone-plate viscometer [9], [10]. Similarly, Chen et al. [11], [12] used image processing method for analyzing aggregation in channel flow. In this research, we improved the method proposed by Jayavanth et al. [13] to analyze aggregation size in the sedimentation set-up.

II. Population Balance Equation

In this work, we propose a population balance method which can be used to numerically investigate formation and breakage of rouleaux in plasma flow. The present study regards single RBC as "elementary particle" and aggregate or rouleaux as "particles" that are able to collide with and adhere to each other under the action of fluid. The aims of this study is to propose an expression for aggregation of elementary particles. These experimental coefficients can be implemented in the blood flow simulations which are based on population balance modeling including [8].

In general, the PBE is a balance equation of the number

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density of one or multiple particle properties. In our application, the particle property would be the diameter of particles (d_p). The PBE in its most general form is given by [14] as:

$$\frac{\partial n(d_p,x,t)}{\partial t}+\nabla_d\cdot\frac{d(d_p)}{dt}n(d_p,x,t) +\nabla_x\cdot u_p n(d_p,x,t)=B(d_p,x,Y,t)-D(d_p,x,Y,t) \quad (1)$$

In this equation $n(d_p,x,t)$ is the number density of particles with diameter of d_p and it is a function of the particle location x and time t. The term $d(d_p)/dt$ is the growth rate of the particle diameter and u_p is the particle velocity. On the right-hand side of the equation, B stands for the birth and D for the death of particles with the diameter of d_p. The dependence on parameter Y is introduced to describe the birth and death rates of particles influenced by the continuous phase. For example, in case of RBCs, becomes plasma shear rate $\dot{\gamma}$, where it has a direct effect on disaggregation of rouleaux. This term can be restated as:

$$B-D=\left[B_B-D_B+B_A-D_A\right] \quad (2)$$

Term B_B indicates the birth of particle due to breakage (disaggregation) of larger particles, D_B indicates the death of particles caused by breakage, B_A indicate the birth of particle resulted by smaller particle aggregation and D_A indicates particles death as they aggregate with another particle. Both birth and death of particles happen each time either of aggregation or disaggregation happens. In (1), $B-D$ can be assumed as a source term which is affected by the break up or aggregation of RBC's cluster in different sizes.

Second term of (1) is ignored due to small or negligible change in volume of particles. Similarly, density of RBC aggregates does not change and therefore the growth rate of the particle is equal to zero. Which results in (3):

$$\frac{\partial n(d_p,x,t)}{\partial t}+\nabla_x\cdot u_p n(d_p,x,t)=B(d_p,x,\dot{\gamma},t)-D(d_p,x,\dot{\gamma},t) \quad (3)$$

Mathematically speaking, (3) have time, space and size as independent variables, hence, we can assume that the size d_p gives an additional dimension to the PBE. Discretizing the PBE in this dimension results in:

$$\frac{\partial n_i}{\partial t}+\nabla u_i n_i=B_{Bi}-D_{Bi}+B_{Ai}-D_{Ai} \quad for \quad i=1,...,N. \quad (4)$$

This approach is called the method of classes in which particles are classified according to their volume or equivalent diameter. Each class i consists of particles with volume of v_i with diameter of d_i. In this equation number density n_i is defined by:

$$n_i=\frac{Count\ of\ Aggregated\ \ Particles\ in\ size\ "i"}{Volume} \quad (5)$$

(4) can be interpreted as a conservation of number density with four source terms B_{Bi}, D_{Bi}, B_{Ai} and D_{Ai} with unit of $m^{-3}s^{-1}$.

The third term on the left-hand side of (1) (second term in (3) and (4)) is implying that particles are entering and exiting the control volume. In Smoluchowski equation [15] it is assumed that there is no particle coming inside or going outside the control volume therefore this term is neglected. Knowing that the birth and death of particles in blood flow simulation only depend on shear rate and time, we can restate (4) as:

$$\frac{\partial n}{\partial t}=B(\dot{\gamma},t)-D(\dot{\gamma},t) \quad (6)$$

Wiegel [16] then suggests following equations for the death and birth of particles:

$$B_A(\dot{\gamma},t)=\frac{1}{2}\sum_{i=1}^{k-1}K_{i,k-i}S_{i,k-i}n_i n_{k-i} \quad (7)$$

$$B_B(\dot{\gamma},t)=\sum_{j=1}^{\infty}F_{k,j}n_{k+j} \quad (8)$$

$$D_A(\dot{\gamma},t)=\frac{1}{2}\sum_{j=1}^{\infty}K_{k,j}S_{k,j}n_j n_k \quad (9)$$

$$D_B(\dot{\gamma},t)=\frac{1}{2}\sum_{i=1}^{k-1}F_{i,k-i}n_k \quad (10)$$

where $K_{i,j}$ is the collision rate of particles in size i with particles in size j. $S_{i,j}$ is the sticking probability indicating the probability of the formation of an $(i+j)$ size after the collision. Thus the product $K_{i,j}S_{i,j}$ is the aggregation rate between a size i and a size j. $F_{i,j}$ is the disaggregation rate, representing the rate at which particles in size i and particles in size j are formed from the breakup of a size $(i+j)$. Combining (6) with (7) to (10) leads to:

$$\frac{\partial n_k}{\partial t}=\frac{1}{2}\sum_{i=1}^{k-1}K_{i,k-i}S_{i,k-i}n_i n_{k-i}+\sum_{j=1}^{\infty}F_{k,j}n_{k+j} -\frac{1}{2}\sum_{j=1}^{\infty}K_{k,j}S_{k,j}n_j n_k-\frac{1}{2}\sum_{i=1}^{k-1}F_{i,k-i}n_k \quad (11)$$

(11) is known as the generalized Smoluchowski Equations [15]. Owens [8] used (11) to find a correlation for macroscopic particle size. He assumed:

$$K_{k,j}S_{k,j} = \Omega_A \quad , \quad F_{k,j} = \Omega_B \tag{12}$$

where Ω_A and Ω_B are macroscopic aggregation and disaggregation rates. He further simplified (11) for macroscopic condition to have:

$$\frac{d(AAS)}{dt} = \frac{1}{2}\Omega_A n_0 - \frac{1}{2}\Omega_B (AAS)^2 + \frac{1}{2}\Omega_B (AAS) \tag{13}$$

where AAS denote the average aggregate size and n_0 is the total number of red blood cells per unit volume and can be calculated using:

$$n_0 = \sum_{k=1}^{\infty} k n_k \tag{14}$$

Red blood cell volume fraction (also called hematocrit) is the volume percentage (%) of red blood cells in blood. If the hematocrit is given, we can similarly find n_0 by multiplying the hematocrit to the volume of a single red blood cell.

Equation (13) is used in this study to find the aggregation rate. Further details on this equation are provided in [8].

III. Experimental Procedure

A. Microchannel Design and Fabrication

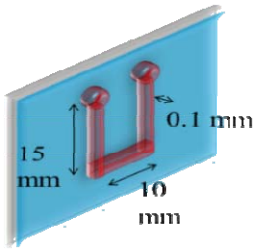

Fig. 1 RBCs sedimentation test microchannel dimensions

To study the sedimentation of RBCs in plasma, a U-shaped channel with 100µm height is used. This channel is similar to the channel proposed in [13] to study the sedimentation of RBCs. The different dimensions of this channel are shown in Fig. 1. Poly-Di-Methyl-Siloxane (PDMS) is used for the fabrication of the channel. The channel is then bonded to a microscope glass slide using oxygen plasma bonding (PE-50 series plasma system, Plasma Etch, USA).

B. Blood Sample Preparation

Healthy human blood is collected into a tube containing EDTA. Samples are centrifuged for 10 minutes, three times at 3000 RPM. After the first round of centrifugation all the plasma is removed from the sample. White blood cells and platelets are discarded after each centrifugation, leaving only the RBCs at the end. Phosphate Buffered Saline (PBS) is added to the RBCs and is gently mixed before the next centrifugation. After the third round, RBCs are suspended in their own native plasma at hematocrits (H_t) of 5%. These hematocrits are checked and confirmed using micro centrifuge before each test.

C. Experiment Setup

The Experimental set-up is shown in Fig. 2. It consists of a high-speed camera (Graftek Imaging, Inc., Austin, TX, USA) controlled using the LabVIEW software (National Instruments, USA), 10x lens magnification and a white light source.

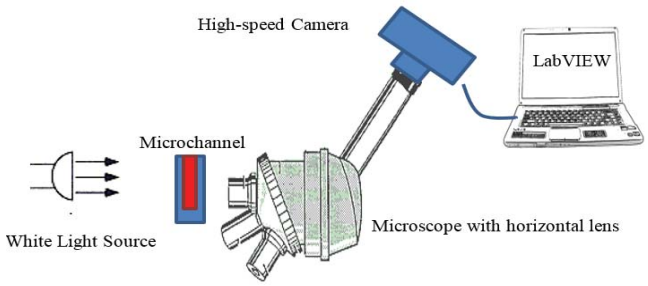

Fig. 2 Video microscopic system used in the sedimentation experiment

Images are recorded at the rate of one frame per second. Via a program developed in LabVIEW, 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.

D. Image Processing Technique

ImageJ software is used to detect the aggregation sizes in the domain. The image is cropped to remove additional information in the picture. To find the background, z-project function [17] is used to find the maximum intensity (background). The background is subtracted and bandpass FFT filtering [18] is applied to the pictures to get a clearer picture of the RBCs. Images are then converted to a binary image. Particle sizes are measured. Resulted images are shown in Fig. 3.

Particle size measurements in this study is done according to Table I.

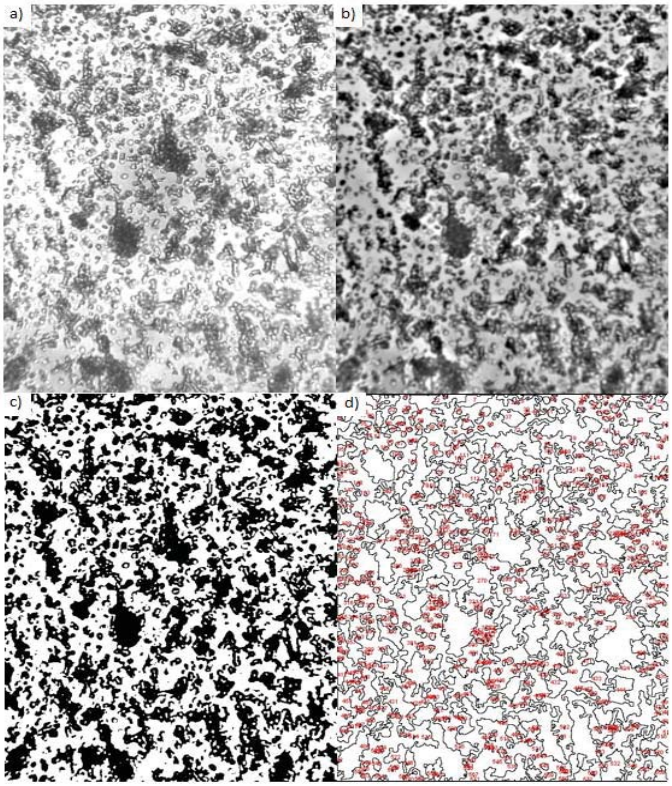

Fig. 3 (a) cropped image, (b) removing the background and filtering, (c) binary image and (d) analyzed picture

TABLE I
PARTICLE SIZES AND VOLUMES

Class i	Number of RBCs	Estimated Volume of particles v_i (μm^3)	Pixel size range	Pictures (not on scale)
1	1	90	25-60	
2	2	180	60-120	
3	4	360	120-360	
4	8	720	360-675	
5	16	1440	675-1500	
6	32	2880	1500-2600	
7	64	5760	2600-5600	
8	128 and more	11520	5600 >	

IV. RESULTS AND DISCUSSION

It is assumed that there is no disaggregation due to low shear rate in this set-up, therefore (13) further simplifies to:

$$\frac{d(AAS)}{dt} = \frac{1}{2}\Omega_A n_0 \tag{15}$$

Average aggregate size (AAS) is found using image processing results for the first 140 seconds. Fig. 4 shows the

average aggregate size (AAS) versus the time and Fig. 4 shows the number density of red blood cells (n_0) during the same time for one sample.

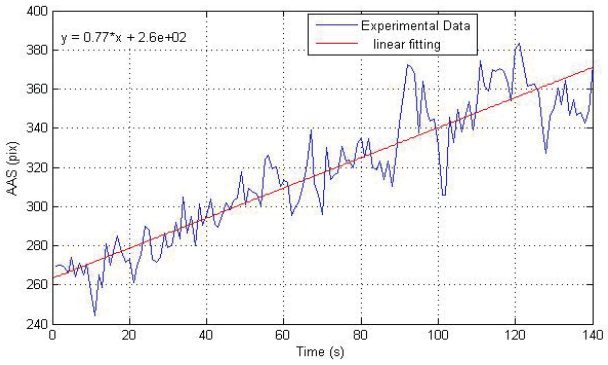

Fig. 4 Change of average aggregate size with time (Sample EU08A)

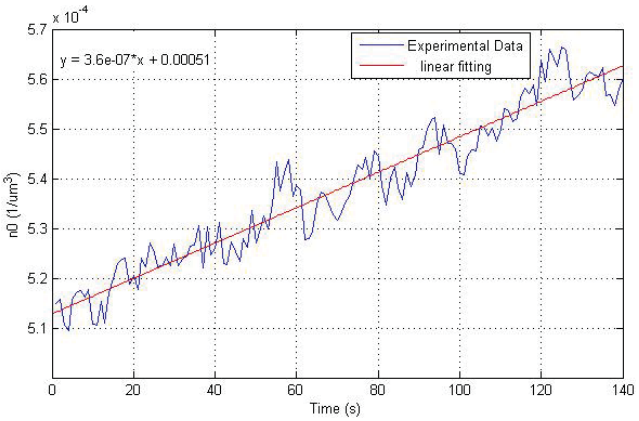

Fig. 5 Change of total number density of red blood cells with time (Sample EU08A)

Applying same method for different samples (5 samples), it is found that $\frac{d(AAS)}{dt} = 0.75 \pm 0.09 \, \mu m^3/s$. Dividing by n_0 it is found that the aggregation rate can be estimated as $\Omega_A = 2.7 \times 10^3 \, 1/s \, (\pm 0.3 \times 10^3)$.

In Figs. 6 and 7, detailed behavior of particles in different sizes is shown. As it can be seen number of particles in small class decrease and number of particles in larger class increases. But middle classes have an increase and then decrease behavior. Decrease of small particles are due to aggregation of particles and results in the increase in larger sizes. Class size 5 and 6 have an increase in their number at the beginning but later they aggregate themselves and decrease.

V. Conclusion

Experiment were designed such that the characteristics of RBCs aggregation can be viewed under a microscope. These experiments were analyzed using image processing techniques to find RBC aggregation sizes in different samples. Using image processing techniques, we were able to analyze the aggregates size distribution in time.

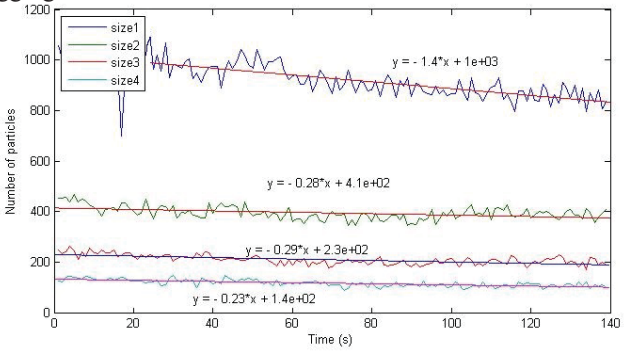

Fig. 6 Change of average aggregate size with time (Sample EU08A)

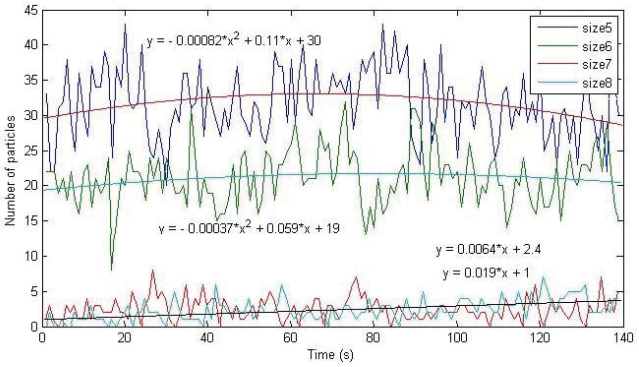

Fig. 7 Change of average aggregate size with time (Sample EU08A)

Ponder [19] suggested that there is a linear relation between the aggregation rate and the hematocrit (constant Ω_A). This relationship can be seen in the experimental data. The aggregation rate found in this study can be called a macroscopic aggregation rate which is different from microscopic aggregation rate. Equation (12) shows that microscopic aggregation rate is the multiplication of the sticking probability with the collision rate. The number of particles in each class as a function of time will be used in further investigation to find these parameters.

To find disaggregation rate as a function of shear rate, a Couette flow set-up will be used to study RBCs under constant shear later in this study. Equation (13) can be simplified for the steady state situation and with the aggregation rate found in this study one can use population balance model to investigate the blood flow.

References

[1] J. K. Wright Chesnutt, "Discrete-Element Model of Red Blood Cell Aggregation in Blood Flow," Ph. D. thesis University of Iowa, Iowa City, IA, 2009.
[2] O. Baskurt, B. Neu and H. J. Meiselman, Red Blood Cell Aggregation, Boca Raton, FL: CRC Press Taylor & Francis Group, 2012.
[3] A. Popel and P. Johnson, "Microcirculation and hemorheology," Annual Review of Fluid, pp. 37(1):43-69, 2005.
[4] H. L. Goldsmith, G. R. Cokelet and P. Gaehtgens, "Robin Fahraeus: evolution of his concepts in cardiovascular physiology," Am J Physiol, vol. 257, p. 1005–15, 1989.
[5] T. M. Geislinger and T. Franke, "Hydrodynamic lift of vesicles and red blood cells in flow — from Fåhræus & Lindqvist to microfluidic cell

sorting," Advances in Colloid and Interface Science, vol. 208, p. 161–176, 2014.
[6] A. M. Robertson, A. Sequeira and R. Owens, "Rheological models for blood," in Hemodynamical Flows: Modeling, Analysis and Simulation, Verlag Italia, Milano, Springer, 2008, pp. 211-241.
[7] M. Bureau, J. C. Healy, J. C. Bourgoin and M. Joly, "Rheological hysteresis of blood at low shear rate," BioRheology, vol. 17, pp. 191-203, 1980.
[8] R. G. Owens, "A new microstructure-based constitutive model for human blood," J. Non-Newtonian Fluid Mech., vol. 140, pp. 57-70, 2006.
[9] T. Shiga, K. Imaizumi, N. Harada and M. Sekiya, " Kinetics of rouleaux formation using TV image analyzer. I. Human erythrocytes," American Journal of Physiology, vol. 245, pp. H252-H258, 1983.
[10] G. Barshtein, D. Wajnblum, and S. Yedgar, " Kinetics of Linear Rouleaux Formation Studied by Visual Monitoring of Red Cell Dynamic Organization," Biophysical Journal Volume 78 2470–2474, May 2000.
[11] S. Chen, G. Barshtein, B. Gavish, Y. Mahler and S. Yedgar, "Monitoring of red blood cell aggregability in a flow-chamber by computerized image analysis," in International and Eighth European Conference on Clinical Hemorheology, Vienna, Austria, 1993.
[12] S. Chen, B. Gavish, S. Zhang, Y. Mahler and S. Yedgar, "Monitoring of erythrocyte aggregate morphology under flow by computerized image analysis," Biorheology, vol. 32, no. 4, pp. 487-496, 1995.
[13] S. Jayavanth and M. Singh, "Computerized analysis of erythrocyte aggregation from sequential video-microscopic images under gravitational sedimentation," ITBM-RBM, vol. 25, no. 2, pp. 67-74, 2004.
[14] D. Ramkirishna, Population Balances: Theory and Applications to Particulate Systems in Engineering, Academic Press, 2000.
[15] M. V. Smoluchowski, "Veruch einer mathematischen theorie der koagulationkinetik kolloider losungen," Z. Phys. Chem., vol. 192, pp. 129-168, 1917.
[16] F. W. Wiegel, “A network model for viscoelastic fluids”, Physica, vol. 42, pp. 156–164, 1969.
[17] B. Forster, D. Van De Ville, J. Berent, D. Sage, M. Unser, "Complex Wavelets for Extended Depth-of-Field: A New Method for the Fusion of Multichannel Microscopy Images," Microsc. Res. Tech., 65(1-2), pp. 33-42, September 2004.
[18] R. C. Gonzalez, R. E. Woods, “Digital Image Processing”, Second edition, Prentice Hall, Upper Saddle River, New Jersey.
[19] E. Ponder, "On sedimentation and rouleaux formation," Q. J. Exp. Physiol., vol. 16, p. 173–194, 1924.