Continuous Magnetic Separation of Blood Components from Whole Blood

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Abstract — Direct magnetic separations of red blood cells from whole blood have been carried out using a continuous magnetic separation method based on high gradient magnetic separation (HGMS) and a gas-permeable membrane with nitrogen gas. The experimental results have shown good agreements with the theoretical model taking into account the gravitational force. Based on the analysis, the feasibility of a direct magnetic separation device for white blood cells and plasma from whole blood is discussed.

I. INTRODUCTION

Fundamental work in the development of magnetic separation of red blood cells has been previously reported [1]-[4]. Most of this work, however, has been done with additives to enhance the magnetic state of red blood cells and to obtain effective magnetic separation. Direct magnetic separation of red and white blood cells from whole blood without any additives has been confirmed in a quasi-static state of very low flow conditions using an optical observation method [5]. In those experiments, the magnetic state of the red blood cells was controlled by passing blood through a gas-permeable tubular membrane with oxygen or nitrogen gas on the outside.

The continuous magnetic separation method [6], [7] provides a very sensitive and selective separation technique. This technique is especially desirable for selective separation between very weakly paramagnetic and diamagnetic particles. This magnetic separation method makes it possible to develop a unique direct separation apparatus for biological cell separation for clinical usage. Biological cells can be separated without the use of additives such as magnetic tagging agents or reducing agents. Thus, blood components separated by this method could be returned to the original or another patient directly after treatment.

Here experimental results of direct red blood cell separation performed using the continuous magnetic separation method and a gas-permeable membrane with nitrogen gas are discussed. The results are compared with a theoretical model taking into account the gravitational force. Based on this analysis, magnetic apparatuses for white blood cell and plasma separations from whole blood are discussed.

Fig. 1 shows a schematic of the continuous magnetic separation process based on high gradient magnetic separation (HGMS). The magnetic separator consists of a long flow channel composed of a thin rectangular cross-section tube and a ferromagnetic wire. The flow channel has multiple outlets which are numbered #1 to #3 from the magnetic wire side. The magnetic field is applied vertically, and parallel to the gravitational force. Paramagnetic particles are attracted toward the magnetic wire while diamagnetic particles are repelled. This geometric configuration has been called the paramagnetic capture mode [6].

Fig. 2 shows a fabricated continuous separator of 3.6 m flow length. The flow channel made from plastic tubing is wound with a ferromagnetic wire in a 14-turn, single-spiral, rectangular-groove on an 82 mm diameter, 100 mm long Plexiglas tube. The flow channel tube has one inlet and three outlets.

II. CONTINUOUS MAGNETIC SEPARATOR

Fig. 1 shows a schematic of the continuous magnetic separation process based on high gradient magnetic separation (HGMS). The magnetic separator consists of a long flow channel composed of a thin rectangular cross-section tube and a ferromagnetic wire. The flow channel has multiple outlets which are numbered #1 to #3 from the magnetic wire side. The magnetic field is applied vertically, and parallel to the gravitational force. Paramagnetic particles are attracted toward the magnetic wire while diamagnetic particles are repelled. This geometric configuration has been called the paramagnetic capture mode [6].

Fig. 2 shows a fabricated continuous separator of 3.6 m flow length. The flow channel made from plastic tubing (1.96 mm OD, 1.47 mm ID) which was co-wound with a ferromagnetic wire (stainless steel SUS 430, saturation magnetization M_s = 1.7 T, radius a = 0.52 mm) in a 14-turn, single-spiral, rectangular-groove (3.48 mm x 1.09 mm) on a 82 mm diameter, 100 mm long Plexiglas tube. The flow channel tube has one inlet and three outlets. This separator provides a paramagnetic particle rich slurry from outlet #1 (the nearest outlet to the magnetic wire) and a diamagnetic particle rich slurry from outlet #3 (the farthest outlet from the magnetic wire).

III. RED BLOOD CELL SEPARATION EXPERIMENT

Human venous blood samples used for the experiment were drawn at a local Medical Department into evacuated
glass tubes containing sodium citrate. The hematocrit (cell concentration) was reduced to about 13% by dilution with the plasma of the drawn blood. To obtain the deoxygenated state of the red blood cells, a gas permeable membrane method was used in the same way as that first demonstrated by single wire HGMS experiments at Purdue University[5]. The blood sample was fed into the magnetic separator through a 1 mm long gas permeable membrane tube of Stilastic® silicon medical-grade tubing (0.5 mm ID, 0.9 mm OD, Dow Corning Co.). Nitrogen gas flow was provided around the permeable membrane tube. The blood flow rate was controlled by a syringe pump. Cell samples recovered from the outlets were evaluated by sedimentation using a glass capillary tube (0.8 mm ID, 1.1 mm OD, and 90 mm long) to determine the red cell concentration. The separator was operated in a magnetic field of 2 T which was generated with a warm-bore superconducting solenoid magnet.

\[ F_0 + F_p + F_d = 0 \]  

![Fig. 3](image-url)  
Fig. 3. Experimental results of red cell separation from whole blood at various flow velocities and at magnetic fields of zero and 2 T. Relative concentrations among three outlets are plotted.

![Fig. 4](image-url)  
Fig. 4. Experimental results of red cell separation from whole blood as a function of magnetic field and flow velocity.

Fig. 3 shows the separation results of red blood cells. Relative concentrations for the three outlets are plotted for the average flow velocities of 6.4 mm/s, 9.5 mm/s and 12.6 mm/s at magnetic fields of zero and 2 T. A similar experimental data of the relative concentrations are shown as a function of the magnetic fields in Fig. 4. The data clearly shows a magnetic field effect on the separation concentration.

IV. THEORETICAL ANALYSIS OF EXPERIMENTAL RESULTS

The separation processes of our continuous separator are analyzed in the same way as in [6]. The gravitational force, however, is not negligible in the present separator configuration shown in Fig. 1, and our analysis, therefore, takes it into account.

The gravitational force on the cell of a volume \( V_p \) is given by

\[ F_g = -g (\rho_p - \rho_f) V_p x \]

where \( x \) is the unit vector in the vertical direction (the direction of the gravitational force), \( g \) is the gravitational constant, and \( \rho_p \) and \( \rho_f \) are the densities of the cells and fluid, respectively. The hydrodynamic drag force at zero flow is given in the Stokes region by

\[ F_d = -6 \pi \eta v_p v \]  

where \( \eta \) is the fluid viscosity, \( b \) is the radius of the cell, and \( v \) is the velocity of the cell. The cell motion in the magnetic separation process is found from the force equilibrium equation

\[ F_0 + F_g + F_d = 0 \]  

where

\[ F_0 = - (\chi_p - \chi_f) a^2 \mu_0 M H_b V_p / x^3 \]  

where \( a \) is the radius of the ferromagnetic wire, \( M \) is the magnetization of the wire, and \( H_b \) is the applied magnetic field, and \( \chi_p \) and \( \chi_f \) are the magnetic susceptibilities of the particles (cells) and fluid (plasma), respectively.

The cell traveling time \( T \) required for the cell to move from the entering position \( x_0 \) to the position \( x_t \) at the separator outlet is obtained from Eq. (1). The time \( T \) should be equal to \( L/v_{0} \) where \( L \) is the separator length, and \( v_{0} \) is the average flow velocity. Consequently, the equation to describe the cell motion through the separator is given as:

\[ L/V_x / (a v_{0}) = G(x_0) - G(x_t), \]  

here

\[ G(x) = x - X_1[(1/6) \log |(x + X_3)(x^2 + X_3)] + 3^{1/2} \arctan[(2x - X_3)/3^{1/2} X_3] \]  

\[ X_1 = (V_{00}/V_{01})^{1/3} = (\mu_0^2 M H_b / (\rho_p a)^{1/3}) \]  

\[ V_{00} = 2 \mu_0^2 M H_b b^3 / (9 \eta a) \]  

\[ \rho = \rho_p - \rho_f \]  

\[ \chi_p - \chi_f \]  

where \( x_{0a} = x_0 / a \) (\( x_0 \) is the entering position), and \( x_{1a} = x_1 / a \) (\( x_1 \) is the position at the outlet.).

The separation capacity of the throughput \( Q \) (“the cross-section” \( x \) “the average flow velocity”) is given by

\[ Q = (x - X_3)(S / a)^{1/2} \sqrt{V_x} / G(x_0) - G(x_t) \]  

where \( S \) is the canister thickness, and \( X_0 \) and \( X_1 \) are the distances from the walls of the canister to the wire axis as shown in Fig. 1. If the separator dimensions and the magnetic field are scaled by the magnetic wire radius \( a \), the throughput \( Q \) is proportional to \( a^{-2} \). It is noted that the throughput is proportional to the particle radius squared, \( b^2 \), since \( V_x \) is proportional to \( b^2 \) and \( G(x) \) is not a function of \( b \). For analytic calculations, the magnetic susceptibilities \( \chi_p = -3.8 \times 10^{-8} \) (in SI units) for deoxygenated red blood cells, \( \chi_f = -7.2 \times 10^{-8} \) for plasma [5], and the relative density \( \rho = \rho_p - \rho_f = 100 \text{ kg/m}^3 \) (\( \rho_p = 1100 \text{ kg/m}^3 \) [8] and \( \rho_f = 1000 \text{ kg/m}^3 \)) were used. The following parameter was chosen for the best fit of the experimental data obtained for the average flow velocities of 6.4 mm/s, 9.5 mm/s and 12.6 mm/s at magnetic fields of zero and 2 T:

\[ b^2 / \eta = 3.57 \times 10^9 \text{ m/kg s} \]  

This value of \( b^2 / \eta \) agrees well with the experimental value obtained from a sedimentation test for the same blood as that used for the magnetic separation experiment.

Fig. 5 shows both calculated and experimental results of the relative concentrations for the three outlets at the flow velocity \( v_0 = 6.4 \text{ mm/s} \). In Fig. 6(a) and (b), calculated results are compared with the experimental results as a...
function of the flow velocity. The figure 6(a) and (b) show the relative concentrations of red blood cells at the magnetic fields of 2 T and zero, respectively. Separation of red blood cells due to the gravitational force can be seen in Fig. 6(b). Overall the experimental results agree well with the results calculated from the equations taking into account the gravitational force.

![Flow Velocity vs. Experimental Results](image)

**Fig. 5.** Experimental results at the flow velocity 6.4 mm/s are plotted with theoretical calculation results.

![Flow Velocity vs. Relative Concentration of Red Blood Cells](image)

**Fig. 6.** Both experimental and theoretical calculation results as a function of the average flow velocities. (a) Magnetic field = 2 T, (b) Magnetic field = 0.

V. SIMULATIONS OF WHITE CELL AND PLASMA SEPARATIONS

White blood cell and plasma separations by the continuous separator are analyzed using Eq. (3) for the present separator shown in Fig. 2. White cells and oxygenated red cells in plasma have shown diamagnetic captures in single wire HGMS experiments [5]. Those experiments indicated that those cells are more diamagnetic than plasma. However, the magnetic susceptibilities of those cells have not been found in the literature. Therefore, we used $\chi_0 = -1.16 \times 10^{-6}$ for both white blood cells and oxygenated red cells for the calculation. This value gives the same absolute value $|\chi_0 - \chi_d|$ as that of the deoxygenated red blood cells, but the sign is negative. White blood cells are classified mainly into two groups and five different kinds of cells, agranulocytes (lymphocytes and monocyte) and granulocytes (neutrophil, eosinophil and basophil). Their sizes are between about 6 µm and 15 µm in diameter. In the simulations, however, the value in Eq. (11) was used for the parameter $b/\eta$.

For white blood cell separation, the ferromagnetic wire is mounted above the flow channel in a horizontal magnetic field as shown in Fig. 7(a). The other configuration shown in Fig. 7(b) is used for plasma separation. In these configurations, the particle separation profiles are calculated from Eq. (3) by replacing Eq. (4) with Eq. (12),

$$G(x) = x + X_0 \left\{ \frac{1}{6} \log \left| \frac{x - X_0}{x_0^3 - X_0^3} \right| - 3 \sqrt{\frac{3}{2}} \arctan \left( \frac{3 + x + X_0}{3 \sqrt{2} x_0} \right) \right\}$$

(12)

Fig. 8 shows relative concentrations calculated for white and oxygenated red blood cells in the diamagnetic capture mode shown in Fig. 7(a). Fig. 8 is also applicable to deoxygenated red cell separation in the paramagnetic capture mode shown in Fig. 7(b). These operating conditions have been called the attractive force mode [6]. On the other hand, Fig. 9 illustrates calculated results for the repulsive force mode which is applicable to deoxygenated red blood cells in the diamagnetic capture mode shown in Fig. 7(a), or white and oxygenated red blood cells in the paramagnetic capture mode shown in Fig. 7(b).

![Continuous Magnetic Separator](image)

**Fig. 7.** Cross-section view of the continuous magnetic separator having the magnetic wire at the top. The magnetic field is applied: (a) horizontally for the diamagnetic capture mode (Inset: Alternate diamagnetic capture method with a vertical field) and (b) vertically for the paramagnetic capture mode. Flow direction is perpendicular to the drawing plane.

A. White Blood Cell Separator

When deoxygenated whole blood continuously passes through the separator in the configuration shown in Fig. 7(a), white-cell rich plasma and red-cell rich plasma are recovered from outlet #1 (the nearest outlet to the wire) and outlet #3 (the farthest outlet from the wire), respectively. The separator having three outlets provides white blood cell rich plasma quantity of one third of the treated blood.

As seen in Fig. 9, deoxygenated red blood cells are completely repelled from the region of outlet #1 at 30 mm/s and magnetic fields greater than 6 T. White cell recoveries at 6 T are 54%, 46% and 41% at the flow velocities of 6.4 mm/s, 15 mm/s and 30 mm/s, respectively, as seen in Fig. 8. At 10 T white cell recoveries can increase to 66%, 55% and 47% at the flow velocities of 6.4 mm/s, 15 mm/s and 30 mm/s, respectively. Those flow rates correspond to the throughput of 33 mL/h, 70 mL/h and 150 mL/h, respectively. The treatment capacities can be increased by increasing the magnetic field and the separator length. From Eq. (10), if the magnetic field and all the separator dimensions including the wire size are doubled, the throughput can be increased by four times with the same flow velocities. If this doubled-dimension separator is extended to 15 m, it might be possible to increase the capacity to 1.2 L/h with white cell recovery of about 50% at the field of 12 T and the average flow velocity of 30 mm/s.
B. Plasma Separator

To separate plasma from whole blood, the continuous magnetic separator can be operated with an oxygenated blood state in the configuration shown in Fig. 7(b). Both red and white blood cells are diamagnetic in oxygenated blood [5]. Therefore, they are repelled from the region of outlet #1 (the nearest outlet to the wire) and plasma without red and white cells is obtained from outlet #1. In this case calculated results of the simulation are shown in Fig. 9. The present separator in Fig. 2 may treat whole blood of 150 mL/h (30 mm/s) at a field of 6 T to separate plasma of 50 mL/h (1/3 of separator in Fig. 2) may treat whole blood of 150 mL/h (30 mm/s) at a field of 6 T to separate plasma of 50 mL/h (1/3 of separator in Fig. 2). The present separator in Fig. 2 may treat whole blood of 150 mL/h (30 mm/s) at a field of 6 T to separate plasma of 50 mL/h (1/3 of separator in Fig. 2). The present separator in Fig. 2 may treat whole blood of 150 mL/h (30 mm/s) at a field of 6 T to separate plasma of 50 mL/h (1/3 of separator in Fig. 2). Therefore, the simulation results are acceptable for the first approximation of white cell and plasma separations. For example, a 15 m long separator with a magnetic wire of 2 mm diameter may perform white blood cell separation at a rate of 1.2 L/h with a white cell recovery of about 50% at a field of 12 T. The recovery can be increased by reducing the separation speed. It may be possible to use the same separator to separate plasma from whole blood at 1.2 L/h and 6 T.

The experiments of red blood cell separation were carried out using blood of hematocrit 13% which was diluted with the plasma. Hematocrit of human blood is approximately 40 – 45%. The separation efficiency decreases with increasing hematocrit. The separation efficiencies could be improved by optimizing the separator design in various ways, such as by using a multi-stage operation and multiple-ferromagnetic-wire arrangements to combine diamagnetic and paramagnetic capture modes.

IV. DISCUSSION AND CONCLUSIONS

A continuous magnetic separator of 3.6 m length was fabricated and used to demonstrate red blood cell separation from whole blood at a magnetic field of 2 T. The experiments of red cell separation were carried out using human venous blood samples diluted to about hematocrit 13% with the plasma of the drawn blood. The experimental results agreed well with the theoretical analyses taking into account the gravitational force. Simulations of white blood cell and plasma separations from whole blood have shown the feasibility of a magnetic separation apparatus for those applications. These simulations of white blood cell and plasma separations were performed with the estimated magnetic susceptibilities \( \chi = -1.16 \times 10^{-6} \) of the white cells and oxygenated red blood cells. To estimate a worse case scenario for white cell separation, we use the magnetic susceptibility of water \((-9.2x10^{-6})\) for white cells. In this case the relative susceptibility \( \chi / \chi_w \) is about half of the value we used for the above simulation. However, white cells are much larger than red blood cells. The volumes of lymphocytes, monocytes and granulocytes are approximately 3, 8, and 6 times the red blood cell volume, respectively [9]. Magnetic force is proportional to the volume of cell. In the continuous magnetic separation process, the throughput \( Q \) is proportional to the particle radius squared, \( b^2 \), as discussed with Eq. (10). These two factors of the magnetic susceptibility and the volume are approximately canceled out. Therefore, the simulation results are acceptable for the first approximation of white cell and plasma separations.

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