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Capillary magnetophoresis of human blood cells and their magnetophoretic trapping in a flow system

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Abstract

The performance of a capillary magnetophoretic device was improved by enhancing the magnetic field gradient using a pair of small iron tips attached to the Nd–Fe–B magnets. The magnetophoretic intensity, $B(\mathrm{d}B/\mathrm{d}x)$, was determined as a function of distance along the gap between the tips from the magnetophoretic velocity of a 3 μm polystyrene microparticle in 0.6 *M* manganese(II) chloride solution. The maximum intensity was increased 4.5 times by the attached iron pieces. The magnetophoresis of a single human blood cell in 0.1 *M* manganese(II) solution was studied by this method and its magnetic susceptibility was estimated. Magnetophoretic trapping of red blood cells was demonstrated under counter-current flow conditions in the capillary.

Keywords: Magnetophoresis; Flow fractionation

1. Introduction

Electrophoresis and sedimentation are widely employed for the separation of biomolecules and cell composites [1,2]. These methods utilize an electric field gradient and a gravitational field, respectively. Though in principle other external fields could also be expected to be utilized for migration analyses of microparticles, they have hardly been applied so far. In the present study, magnetophoresis, the migration of particles in an inhomogeneous magnetic field, was investigated. Recently, some magnetic separation methods have been reported [3]. In these methods, particles, magnetically modified by the adsorption of paramagnetic ions or fine paramagnetic particles, were separated using the magnetic force generated by an inhomogeneous magnetic field. However, biological particles are usually diamagnetic. Therefore, another method is needed to separate diamagnetic biological particles. The magnetic separation of diamagnetic particles in paramagnetic aqueous media has been studied for industrial purposes using packed stainless steel wire [4]. Magnetophoretic behavior of diamagnetic cells in water has been observed under a magnetic field gradient [5]. We have recently studied the magnetophoretic behavior of a single polystyrene particle [6] and a single paramagnetic droplet [7] in a square silica capillary under a well-defined high magnetic field gradient, on the micrometre scale, produced by a pair of rare earth magnets. Under these conditions, the magnetic buoyancy in the paramagnetic media dominated the force acting on the particles. We also suggested in the previous report the possibility of magnetophoretic trapping fractionation of microparticles with various sizes. In this method, the migration velocity is governed by the magnetic field gradient. Therefore, the enhancement of the gradient is an important factor for the improvement of magnetophoresis.

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The aim of this work is to improve the magnetophoretic method by enhancing the magnetic gradient in small Nd–Fe–B magnets and to apply it to the magnetophoresis and magnetophoretic trapping of red blood cells.

2. Magnetophoresis and magnetophoretic trapping

In an inhomogeneous magnetic field, a particle in a solution experiences two types of forces. One is the magnetic force working on the particle itself, F_p, and the other is the magnetic buoyancy, F_f, exerted by the paramagnetic medium, with a direction opposite to F_p. The net force working on a particle, F_m, can be described as follows

$$F_m = F_p - F_f = \frac{V(\chi_p - \chi_f)}{\mu_0}\left(B\frac{dB}{dx}\right) \quad (1)$$

where V is the volume of the spherical particle (m^3), χ_p and χ_f are the respective magnetic susceptibilities per unit volume of the particle and the medium, μ_0 is the vacuum magnetic permeability (NA^{-2}) and dB/dx the gradient of magnetic flux density ($NA^{-1}m^{-2}$). Acceleration and mass of the particle are so small in our system that the particles can migrate with a constant velocity, v, keeping balance between the net magnetophoretic force, F_m, and the viscous or frictional force, F_v, which is expressed by Stokes' law

$$F_v = 6\pi\eta r v \quad (2)$$

where η is the fluid viscosity (Pa s) and r the radius of the spherical particle (m).

From Eqs. (1) and (2), we can obtain the migration velocity v of a spherical particle as

$$v = \frac{2}{9}\frac{(\chi_p - \chi_f)r^2}{\mu_0\eta}\left(B\frac{dB}{dx}\right) \quad (3)$$

This equation can be used for the determination of $B(dB/dx)$ on the micrometre scale from the measurement of the velocity of a microparticle as a function of x. Also, at the point of minimum $B(dB/dx)$, a microparticle can be trapped in the capillary with the force of F_m. In addition, by controlling the rate of counter-current flow against the magnetophoretic buoyancy, differently sized microparticles can be segregated. If the flow-rate is lower than the velocity represented by Eq. (3), the particle cannot go through the minimum point and is trapped. When the flow-rate is the same as the velocity v, the probability of the particle passing through the minimum point is 50%. A flow-rate higher than v will result in complete passing.

3. Experimental

3.1. Samples

The diameter of the polystyrene latex particles used in the experiments was 2.77 ± 0.5 μm (Polybead-Polystyrene, Funakoshi, Tokyo, Japan) and its magnetic susceptibility was -8.21×10^{-6}. The paramagnetic electrolyte solution used as medium was 0.6 M or 0.1 M $MnCl_2$, which was GR reagent of Katayama Kagaku. The magnetic susceptibilities of 0.6 M and 0.1 M $MnCl_2$ per unit volume were determined to be 1.036×10^{-4} and 1.054×10^{-5} by a magnetic balance (MSB-AUTO, Sherwood Scientific, UK). These values were much larger than those of the polystyrene particles. Consequently, polystyrene particles could be migrated by the magnetic buoyancy. The density of polystyrene particles (1.05 g cm^{-3}) was almost the same as that of 0.6 M $MnCl_2$. The viscosity of 0.6 M $MnCl_2$ was 1.12×10^{-3} Pa s.

Fresh human blood was sampled just prior to use, from the author into a vial that contained EDTA aqueous solution to prevent the aggregation of the cells. One drop of blood sample was added to 10 ml of 0.1 M manganese(II) chloride solution, which is almost isotonic with real blood.

3.2. Apparatus

The square fused-silica capillary (Polymicro Technologies, Phoenix, AZ) used for magnetophoresis had a 100 μm×100 μm inner section and was 10 cm long. The sample solution was introduced into the capillary and both ends of the capillary were sealed by vacuum grease. In the experiment of counter-current trapping, the solution flowed via a syringe

(Hamilton, Gastight, No.1701, Reno, NV) and a pump (Pump 11, Harvard Apparatus, Holliston, MA). The flow-rate was varied in the range of 0.3–3.0 μl h^{-1}.

The magnetic field was enhanced by iron (99.8%) pieces (3 mm height×1 mm width×7 mm long) on each rare earth magnet (Nd–Fe–B, Neomax, Sumitomo Special Metals, Tokyo, Japan) as shown in Fig. 1. The size of the magnet was 17 mm×19 mm×3 mm. A small aluminum block kept the distance between the two iron pieces at 400 μm. The configuration of the magnets shown in Fig. 1 generated a large magnetic field gradient along the x-axis and enabled the magnetophoresis of polystyrene microparticles in manganese(II) solution. The edge of the iron tips was defined as $x=0$ and the direction toward the interior of the gap between the tips as $x>0$. An optical microscope (Chuo Seiki, Japan) with a CCD camera (CN42H, ELMO, Nagoya, Japan) was used to observe the migration of particles in the range of $-800\ \mu m<x<1000\ \mu m$.

Fig. 2 shows a schematic diagram of the experiment of counter-current flow trapping. When a sample solution flowed in the positive direction in the capillary, a strong force of opposing buoyancy was exerted on the particles. The region from −90 μm to 350 μm was defined as the observed region. In case of low flow-rates, the particles were trapped in the observed region. However, the particles passed through the observed region when the flow-rate was high enough. To investigate the efficiency of magnetic trapping, trapping efficiency was defined as

$$\langle \text{Trapping efficiency} \rangle = \frac{N_1 - N_2}{N_1} \times 100\ (\%) \qquad (4)$$

where N_1 is the number of particles entering the observed region and N_2 is the number of particles escaping from the observed region. We measured the trapping efficiency of red blood cells as a function of the flow-rate in the 0.1 M $MnCl_2$ solution.

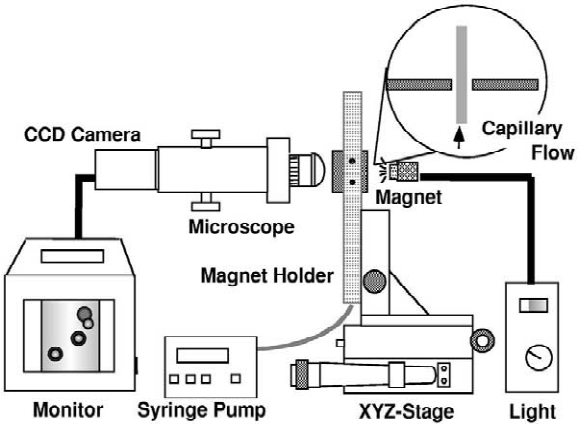

Fig. 2. Schematic drawing of the apparatus for the counter-current magnetophoretic trapping of a single red blood cell.

4. Results and discussion

4.1. Magnetophoresis of polystyrene particles

Fig. 3a shows the x-component of migration velocity for a 3 μm polystyrene particle in the region of $-800\ \mu m<x<1000\ \mu m$. The sign of velocity indicates the direction of migration; negative velocity in the region of $x<500$ μm refers to migration toward the negative direction on the x-axis. On the other hand, positive velocity in the region of $x>500$ μm corresponds to migration in the positive direction. This means that any particle in 0.6 M manganese(II) chloride moves out of the gap by magnetophoretic buoyancy. The magnetophoretic inten-

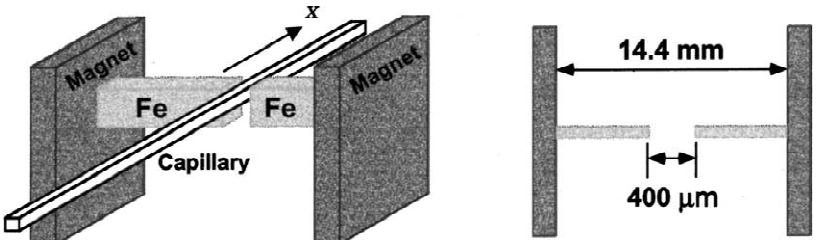

Fig. 1. Enhancement of the magnetic field gradient by the pair of iron pieces attached to Nd–Fe–B magnets.

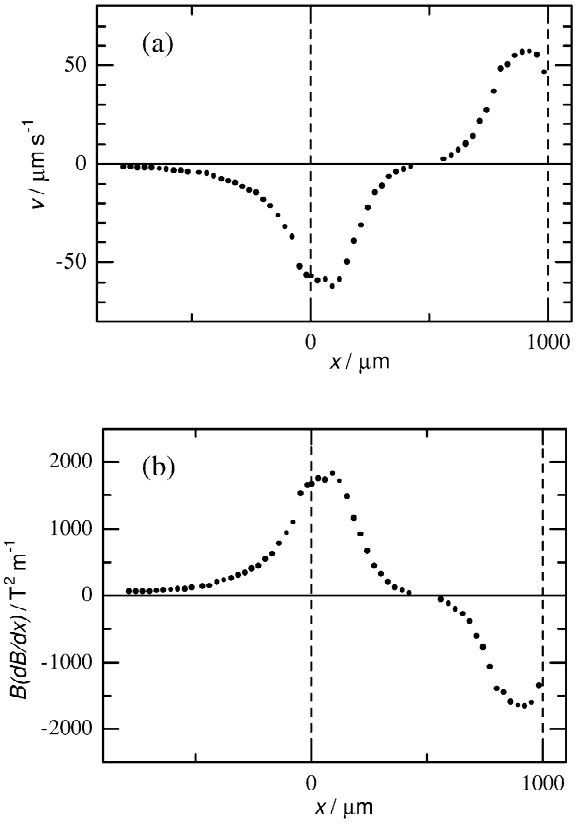

Fig. 3. (a) Magnetophoretic migration velocity of a 3 μm polystyrene particle in 0.6 *M* aqueous manganese chloride in the enhanced magnetic field. (b) Magnetophoretic intensity, $B(\mathrm{d}B/\mathrm{d}x)$, in the enhanced magnetic field determined by the migration velocity of a 3 μm polystyrene particle.

sity, $B(\mathrm{d}B/\mathrm{d}x)$, was obtained from the velocity of a 3 μm polystyrene particle according to Eq. (3). The result is shown in Fig. 3b. The maximum value of $B(\mathrm{d}B/\mathrm{d}x)$ was 1800 $\mathrm{T}^2\ \mathrm{m}^{-1}$, which was 4.5 times larger than the value produced at the edge of the magnets, in the absence of iron pieces, with a 400 μm gap. Again, the maximum and minimum points observed in $B(\mathrm{d}B/\mathrm{d}x)$ around $x=0$ μm and $x=1000$ μm, respectively, indicate that a maximum and equal force is acting on the 0.6 *M* manganese solution toward the center of the gap. This kind of profile in $B(\mathrm{d}B/\mathrm{d}x)$ could be estimated by a simulation software [8].

4.2. Magnetophoresis of red blood cells

The migration velocities of red blood cells are

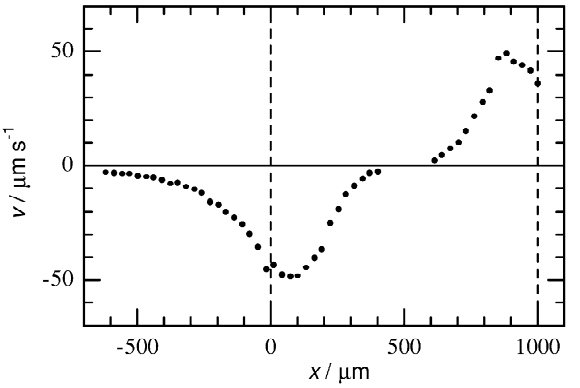

Fig. 4. Magnetophoretic velocities of red blood cells in 0.1 *M* aqueous manganese chloride in the enhanced magnetic field.

plotted in Fig. 4 against the *x*-axis. A red blood cell is a diamagnetic particle [9,10], therefore it migrates by a magnetophoretic buoyancy just as a polystyrene particle does. The profile in Fig. 4 is similar to that in Fig. 3a. If the relation between the observed velocity of a red blood cell and the magnetophoretic intensity $B(\mathrm{d}B/\mathrm{d}x)$ is governed by Eq. (3), we can estimate the volume magnetic susceptibility of a red blood cell from the proportionality coefficient. The observed velocity was plotted in Fig. 5 against $B(\mathrm{d}B/\mathrm{d}x)$ determined from the velocity of polystyrene particles (Fig. 3b). The plot in Fig. 5 gave a straight line with a slope of -2.72×10^{-8} ($\mathrm{m}^2\ \mathrm{s}^{-1}\ \mathrm{T}^{-2}$). The slope corresponds to $2(\chi_\mathrm{p}-\chi_\mathrm{f})r^2/9\mu_0\eta$ in Eq. (3). For the estimation of χ_p from the slope, we need the

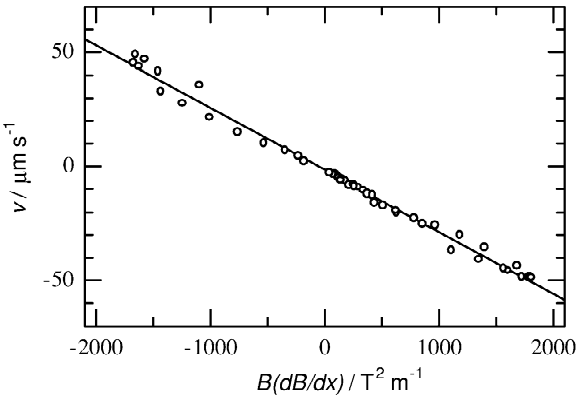

Fig. 5. Correlation between the velocity of red blood cells and the magnetophoretic intensity, $B(\mathrm{d}B/\mathrm{d}x)$. The slope of the line was used for the estimation of the volume magnetic susceptibility of red blood cells.

value of the radius of a red blood cell. A real red blood cell is not a sphere, so we calculated a hypothetical radius, r', of a red blood cell by the equation [10]

$$r' = (3lV/8A)^{1/2} \quad (5)$$

where l is the characteristic length of the particle in the direction of the velocity and A the maximum cross section area perpendicular to the velocity. A/l was used as 5.5×10^{-6} m [11]. If we assume that the volume of a red blood cell in plasma is 8×10^{-17} (m^3), $r' = 2.3\times10^{-6}$ (m) and $\chi_p = -17\times10^{-6}$ can be obtained. This value seems too large as χ_p for red blood cells. Melville et al. used $r' = 4.25\times10^{-6}$ (m) [9]. In this case, $\chi_p = -3.1\times10^{-6}$. It is known that oxyhaemoglobin is diamagnetic while deoxyhaemoglobin is paramagnetic. The magnetic susceptibility of plasma is about -7.7×10^{-6} [10]. The value for a red blood cell in the completely deoxygenated state is $\chi_p = 3.88\times10^{-6}$ [9]. Thus, the values estimated from our experimental results indicated that our sample was a mixture of both types of cells, oxygenated and deoxygenated ones.

4.3. Magnetophoretic trapping of red blood cells

Fig. 6 shows a typical magnetophoretic trapping of red blood cells in the counter-current flow mode. Two red blood cells indicated by arrows introduced downward were trapped in the observed region where $B(dB/dx)$ had a maximum value. Some particles were adsorbed on the wall of the capillary. Every flowing particle was trapped in the observed region when the flow-rate was less than 1.0 μl h^{-1}. As the flow-rate was increased, the cells escaped from the observed region. Finally, with a flow-rate higher than 3 μl h^{-1}, all cells passed through the

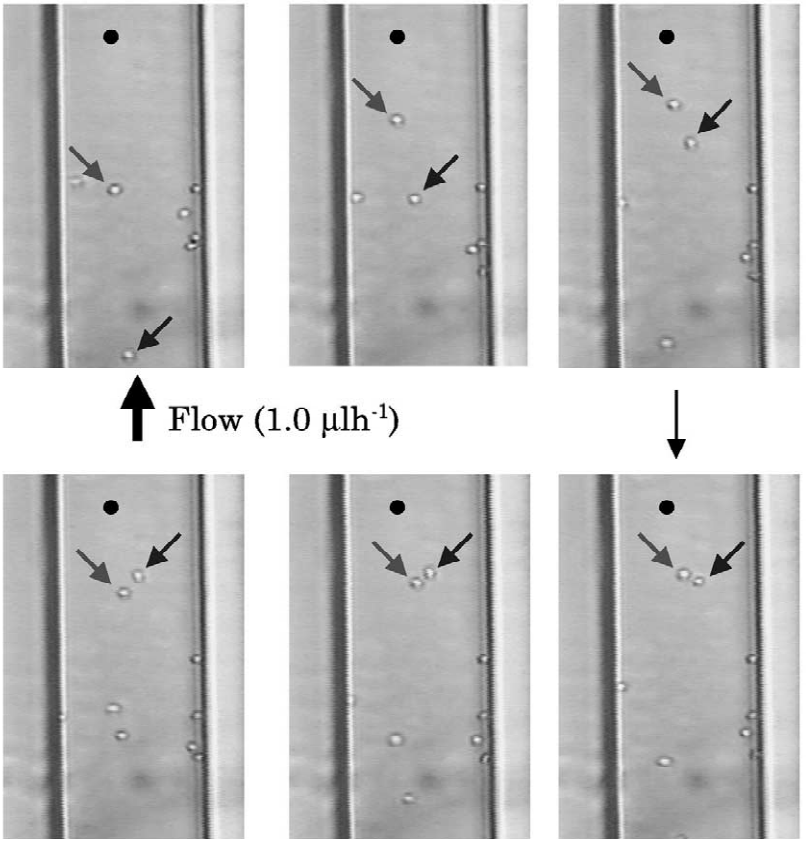

Fig. 6. Photographic representation of the magnetophoretic trapping of red blood cells at a flow-rate of 1.0 μl h^{-1} under the enhanced magnetic field. Pictures are shown at 3-s intervals from panels 1 to 6.

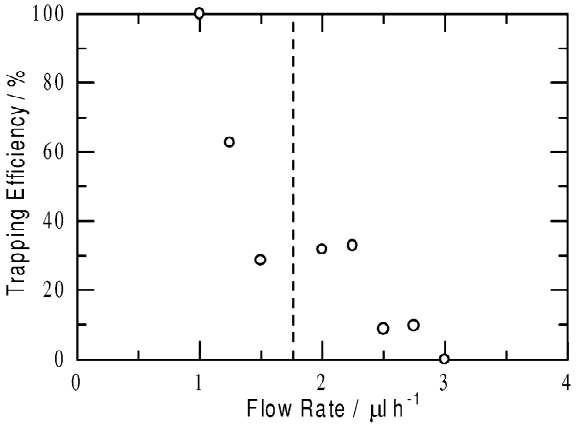

Fig. 7. The efficiency of magnetophoretic trapping of human red blood cells as a function of the flow-rate of 0.1 *M* manganese(II) chloride solution.

observed region. The critical flow-rate expected from Eq. (3) is in good agreement with the threshold flow-rate in Fig. 7, though laminar flow in the capillary resulted in a diffuse threshold flow-rate value. The red blood cell is the largest particle in plasma. Therefore, other smaller particles such as white blood cells and platelets were thought to be removed with a flow-rate of 1.0 $\mu l\ h^{-1}$.

The present study proposed a simple but very effective method to fractionate biological particles. A unique advantage of the magnetophoretic fractionation for biological samples is that there is no heat generation, which may damage the sample. This technique can be extended to the fractionation of smaller particles by using a super-conducting magnet.

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