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Determination of magnetic susceptibility of various ion-labeled red blood cells by means of analytical magnetapheresis

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Abstract

Analytical magnetapheresis is a newly developed technique for separating magnetically susceptible particles. The magnetically susceptible particles are deposited on a bottom plate after flowing through a thin (<0.05 cm) separation channel under a magnetic field applied perpendicular to the flow. Particles with various magnetic susceptibilities can be selectively deposited and separated by adjusting the applying magnetic force and flow rates. Magnetic susceptibility is an important parameter for magnetic separation. Magnetic susceptibility determination of various ion-labeled red blood cells (RBCs) using analytical magnetapheresis with a simple theoretical treatment is reported in this study. Susceptibility determination is based on the balance between maximal channel flow rate and magnetically induced flow rate for deposition. We tried a new approach to determine particle magnetic susceptibilities using a balance of magnetic and drag forces to control magnetically induced particle velocities. The Er^{3+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+} ions were used to label RBC at various labeling concentrations for susceptibility determination. The susceptibilities determined for various ion-labeled RBC under two magnetic field intensities fell within a 10% range. The average viabilities of various ion-labeled RBCs were 96.1 ± 0.8%. The susceptibility determination generally took less than 10 min. Determined susceptibilities from analytical magnetapheresis differed by 10% from reference measurements using a superconducting quantum interference device (SQUID) magnetometer. The cost and time for analysis is much less using analytical magnetapheresis. This technique can provide a simple, fast, and economical way for particle susceptibility determinations.

Keywords: Cells; Magnetic separation; Analytical magnetapheresis

1. Introduction

Magnetic separation and related techniques have been widely used in the manufacturing and mining industries for a long time and have recently been used in wastewater treatment, biotechnology and related applications [1–15]. The advantages of magnetic separation are simplicity, speed, and selectivity. Magnetic separation using permanent magnets as magnetic field sources offers additional economic advantage and developing it further deserves our attention.

Analytical magnetapheresis is one newly developed technique for analyzing magnetic particles using permanent magnets as magnetic field sources [1–3]. In analytical magnetapheresis, magnetically susceptible particles in carriers flow through thin (<0.05 cm), ribbon-like separation channels at controlled flow rates under magnetic fields and form deposition patterns on the channel bottom plate. Front and side views of an analytical magnetapheresis system are shown in Fig. 1. The applied magnetic forces act perpendicular to the channel flow axis and drive magnetic particles toward interpolar gaps. Particles with high field-induced velocity (shown as solid circles in the figure) are attracted by the magnetic forces and deposited upon the interpolar gap as they pass along the separation channel. Particles with low field-induced velocity (shown as hollow circles in the figure) are relatively less affected by the magnetic forces and all exit the separation channel. Therefore, particles with different magnetic susceptibilities are separated into deposited and collected fractions after passing through the separation channel. Particles depositions are calculable from the principle since relevant forces, channel flow rates, and experimental parameters are known. Given the unpacked separation

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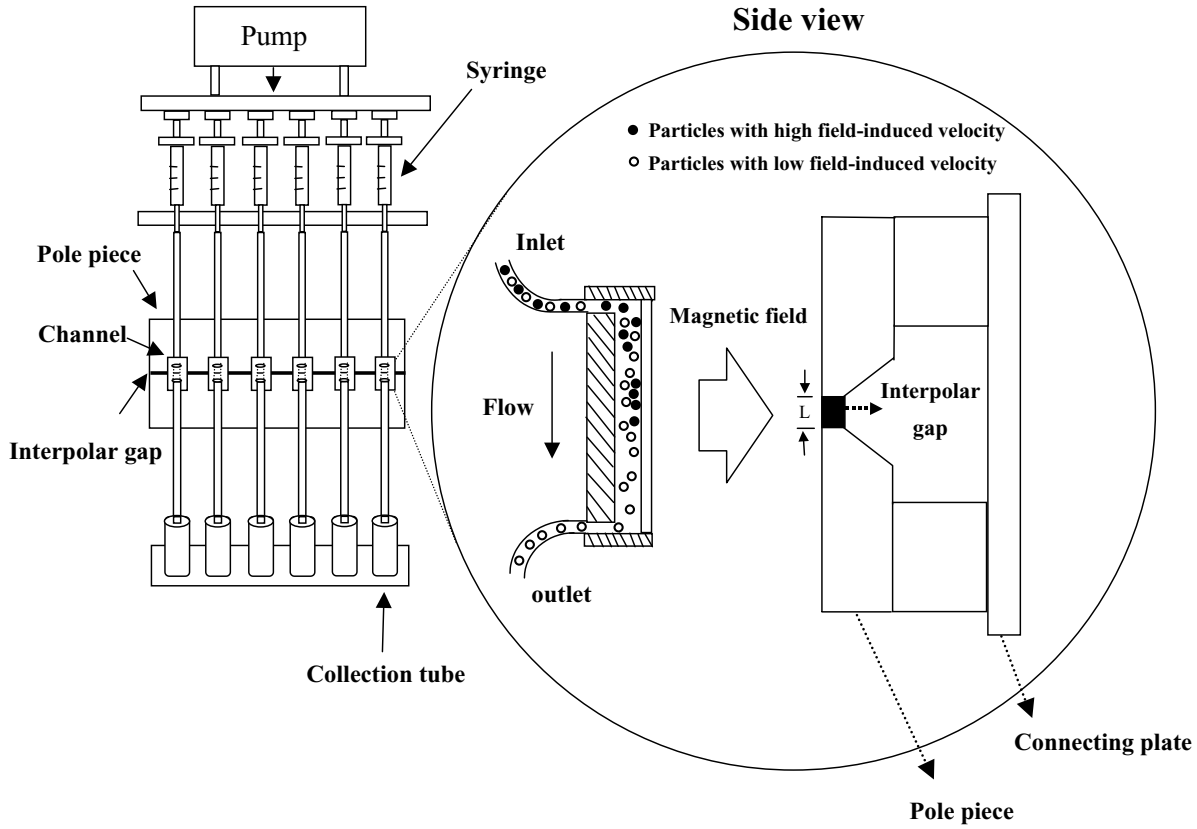

Fig. 1. General schematic of the analytical magnetapheresis system with multiple channels.

channel and its simple geometry, the forces acting on samples can be calculated with good accuracy using known sample physical parameters and magnetic field intensity. One of the physical parameters can also be deduced from the particle depositions with a simple theoretical treatment.

Magnetic susceptibility is an important parameter for magnetic separation. We tried a new approach to determine particle magnetic susceptibilities using a balance of magnetic and drag forces to control magnetically induced particle velocities. Red blood cells (RBCs) labeled with various ions at various concentrations were used to study susceptibility determinations under two field intensities.

2. Theory

Calculating particle magnetic susceptibilities using analytical magnetapheresis with a simple theoretical treatment is summarized in this study. The particles in this separation system were mainly subjected to magnetic force, drag force, gravitational force, and Brownian motion. The gravitational force was parallel to the channel flow and was negligible in this separation system, and Brownian motion is negligible for the micron-sized particles used in this study, therefore, we focused on the magnetic and drag forces in this separation system.

Magnetically induced particle velocity is derived from the balance of magnetic force and drag force, magnetic force (F_m) in the cgs system can be expressed as [3,4]:

$$F_m = \frac{1}{2}\Delta\chi V \nabla B^2 \quad (1)$$

where $\Delta\chi = \chi_p - \chi_c$, χ_p and χ_c are the respective magnetic susceptibilities of particle and carrier, V is the particle volume (for spherical particle $V = (1/6)\pi d^3$, d is the particle diameter), ∇ is the gradient operator, and B is the magnetic field intensity.

Drag force (F_d) can be expressed as:

$$F_d = 3\pi\eta d U \quad (2)$$

where η is the carrier viscosity, d is the particle diameter, and U is the particle migration velocity.

Therefore, magnetically induced particle velocity (U_m) can be obtained by balancing the magnetic force and drag force from Eqs. (1) and (2):

$$U_m = \frac{d^2}{36\eta}\Delta\chi \nabla B^2 \quad (3)$$

For particles to be deposited on the channel bottom plate, the magnetically induced flow rates (bLU_m) must be larger than or equal to the channel flow rates ($\dot{V}_c$), i.e.

$$bLU_m \geq \dot{V}_c \quad (4)$$

where b is the channel breadth and L is the interpolar gapwidth.

Channel flow rates were increased progressively to maximum under complete deposition conditions in order to calculate particle susceptibilities. The maximal channel flow rate ($\dot{V}_{max}$) for complete particle deposition was set equal to the magnetically induced flow rate, as expressed in the following equation:

$$bLU_m = \dot{V}_{max} \quad (5)$$

Substituting Eq. (3) into Eq. (5) yields:

$$\Delta\chi = \frac{36\eta\dot{V}_{max}}{bLd^2\nabla B^2} \quad (6)$$

We can calculate the particle magnetic susceptibility from the known carrier susceptibility and other given experimental parameters (b, L, η, d, $\dot{V}_{max}$, ∇B^2) in the study.

3. Experimental

The channel length, breadth, and thickness used were 1.0, 0.1, and 0.025 cm, respectively. The calculated void volume was 0.0025 ml. The particle concentration in the injected sample suspension was 2.0×10^4 particles/ml. The injection volume was equal to 0.02 ml. The channel assembly was the same as that used in previous works [1,2]. The channel consisted of one cut-out layer of Mylar sandwiched between a plastic sheet and a glass plate, which served as the channel walls. The bottom plate, made of thin (150 μm) glass, was used for particle deposition. All these layers were held firmly together with silicone sealant by pressing them evenly with clamps.

Magnetic fields were generated by a permanent magnet assembly consisting of one pair of rare earth magnets (Nd–Fe–B) connected by soft-iron pole pieces, which conducted the magnetic flux lines to the interpolar gap. The Nd–Fe–B (Neodymium–iron–boron) magnets, characterized by a maximum energy product of 3.5×10^7 G Oe, were obtained from Super Electronics (Taipei, Taiwan). Two gap widths of 1.5 and 2.5 mm and corresponding to the deposition boundary were used for all experiments. The gap length was 10 cm. Magnetic field measurements were made using a Gaussmeter and a Hall-effect probe (Model Gauss 5080, F.W. Bell, Orlando, FL, USA) with adjustable microstages. The probe measured magnetic flux perpendicular to a sensing area with a diameter of 0.4 mm. The combined magnets and pole pieces were 17.5 cm × 10 cm × 6.0 cm and weighed 5.5 kg. The saturation fields (B_0) were 2.29×10^4 and 1.77×10^4 G for respective gapwidths of 1.5 and 2.5 mm.

Light microscopy (Olympus BX-50, Tokyo, Japan) was used for particle counting and verification. A 3% error rate was assumed in complete-deposition particle counting experiments. A multichannel syringe pump (Model 200, KD Scientific Inc., Boston, MA) was used for sample and carrier delivery. A hemacytometer was used to count particles exiting at the outlet. Phosphate-buffered saline (PBS) solutions and Hank balanced salt (HBS) solutions with pHs of 7.02, viscosities η equal to 1.0×10^{-2} g cm^{-1}s^{-1} were used as carriers in this study. Trypan blue, cationized ferritin, manganese sulfate and iron nitrate were purchased from Sigma (St. Louis, MO, USA), copper chloride and nickel chloride were obtained from Aldrich (Milwaukee, WI, USA), erbium chloride was obtained from Strem (Newburyport, MA, USA), M-450 Dynabeads of 4.5 μm size were obtained from Dynal (Lake Success, NY, USA). Blood cells were obtained from the Jen-Ai Hospital in Dali (Taichung County, Taiwan).

Fresh red blood cells from hospital were centrifuged at $50 \times g$ for 5 min to remove plasma. The cells were then washed with PBS and centrifuged three times before labeling. Various labeling ion concentrations were obtained by diluting 100 mM prepared stock solutions. Various ion-labeled RBCs were prepared by mixing 1 ml of labeling ions at fixed concentrations with 9 ml solutions containing 4.0×10^5 RBCs and incubatng in ice for 30 min with shaking every 10 min. All ion-labeled particles were washed three times with HBS solution before use to remove unlabeled ions. The labeling ions used in this study were Er^{3+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Co^{2+}, and Cu^{2+}. Deoxy RBC were prepared by mixing 9 ml solutions containing 2.7×10^4 RBC with 1 ml of 30 mM sodium nitrite at room temperature for 30 min then washing the resulting solutions three times with HBS solution and incubating at 4 °C for 1 h before use. Dye exclusion testing was carried out using trypan blue stain and a hemacytometer. This method was based on the assumption that viable cells did not take up dyes, whereas nonviable cells did. For viability testing, 0.5 ml containing 1.0×10^6 cell suspensions were mixed thoroughly with 0.5 ml of 0.4% (w/v) trypan blue solution for 5 min before counting. The minimal numbers of labeled ions per particle required for complete deposition were calculated by dividing the total numbers of ions by the total numbers of particles and assuming complete labeling efficiency.

Reference magnetic susceptibility measurements were made using an MPMS5 model superconducting quantum interference device (SQUID) magnetometer from Quantum Design (San Diego, CA, USA). Magnetic field strengths from 1.0×10^4 to 2.0×10^4 gauss were used for susceptibility measurements in SQUID. The cgs system and volume magnetic susceptibility, χ, are used throughout this study for convenient calculation unless otherwise indicated.

Magnetapheresis was performed in the following steps. (1) Separation channels were put on the interpolar gap of pole pieces, as shown in Fig. 1. (2) Collection tubes were put at the ends of channel outlets. (3) Syringes with fixed sample volumes were put into syringe pump. (4) The microsyringe pump was turned on for magnetapheresis.

4. Results and discussion

Magnetically induced particle velocity is an important factor for magnetic susceptibility determination using

analytical magnetapheresis with a simple theoretical treatment. Previously, magnetically induced particle velocity was calculated with the assumption that magnetic force was acted on the cross-section area from particle projection [1,2]. The resulting magnetically induced particle velocity was shown to be proportional to the susceptibility difference between sample and carrier, to the particle diameter, to the drop in magnetic field strength square, and inversely proportional to the carrier viscosity. We used the balance between magnetic forces and drag forces without cross-section projection assumption in this new approach for determination of magnetically induced particle velocity, therefore, magnetic force was acted on the whole particle volume. The resulting magnetically induced particle velocity from the new approach was shown to be proportional to the susceptibility difference between sample and carrier, to the particle diameter square, to the gradient of magnetic field intensity square, and inversely proportional to the carrier viscosity.

Particles with high magnetic susceptibility, such as Dynabeads, were used to test this new field-induced velocity approach for susceptibility determination using analytical magnetapheresis at two magnetic field intensities, as shown in Table 1. Determined Dynabeads susceptibilities were consistent with relative standard deviations (R.S.D.) within 9% variation and differed by 5.6–9.4% from the reference SQUID measurement. Particles with low magnetic susceptibility, such as ferritin-labeled RBCs and deoxy RBCs, were also used to test susceptibility determination. These results are also shown in Table 1. Experimentally determined susceptibilities of these low-susceptibility RBCs were consistent with R.S.D. within 11% variation and differed by 5.0–9.8% from the reference SQUID measurement. The determined susceptibilities of high- and low-susceptibility particles using analytical magnetapheresis with this new approach to magnetically induced particle velocity were consistent with R.S.D. within 11% variation and differed by less than 10% from the reference SQUID measurement. This susceptibility determination generally took less than 10 min.

The labeling ions used for RBC susceptibility determination study were Er^{3+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+}. The average viabilities of these ion-labeled RBCs were $96.1 \pm 0.8\%$ based on the dye exclusion test. Labeling concentrations of various ions from 0.1 M to 10^{-7} mM were studied. For all labeling ions, determined ion-labeled RBC susceptibilities were roughly related with their labeling ion concentrations. Higher labeling-ion

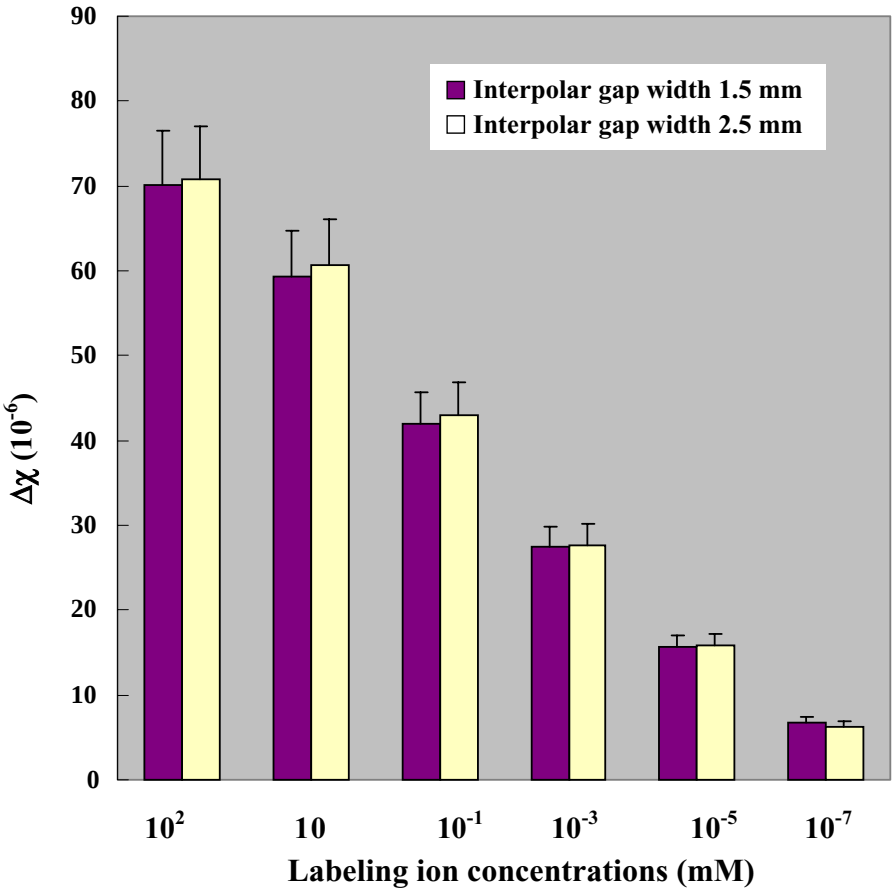

Fig. 2. Determined susceptibilities for various concentration Er^{3+}-labeled red blood cells at two magnetic field intensities. $\nabla B^2 = (1.50 \pm 0.05) \times 10^6$ and $(5.65 \pm 0.16) \times 10^5$ $G^2/\mu m$ were used with respective interpolar gap widths of 1.5 and 2.5 mm.

Table 1
Determined magnetic susceptibilities of particles and labeled red blood cells

Sample	Interpolar gap width (mm)	$\dot{V}_{max}$ (ml/min)	$\Delta\chi \pm$ S.D. (10^{-6}) (cgs) ($n = 3$) (R.S.D.)	SQUID $\Delta\chi \pm$ S.D. (10^{-6}) (cgs) ($n = 10$) (R.S.D.)
Dynabeads[a]	1.5	0.124 ± 0.003	21130 ± 1830 (8.7)	20000 ± 780 (3.9)
	2.5	0.112 ± 0.003	21500 ± 1880 (8.7)	
Ferritin (10^{-5} mM) labeled RBCs[b]	1.5	0.121 ± 0.004	12.9 ± 1.2 (9.3)	11.81 ± 0.39 (3.3)
	2.5	0.073 ± 0.001	12.4 ± 1.1 (8.9)	
Deoxy RBCs	1.5	0.023 ± 0.001	2.45 ± 0.25 (10)	2.72 ± 0.12 (4.4)
	2.5	0.015 ± 0.001	2.55 ± 0.28 (11)	

The values in parentheses are in percent.
[a] Interpolar gap width = 2.5 mm, $\nabla B^2 = 500 \pm 10\,G^2/\mu m$, interpolar gap width = 1.5 mm, $\nabla B^2 = 939 \pm 15\,G^2/\mu m$.
[b] $\nabla B^2 = (1.50 \pm 0.05) \times 10^6$ and $(5.65 \pm 0.16) \times 10^5\,G^2/\mu m$ for respective interpolar gap widths of 1.5 and 2.5 mm.

concentrations qualitatively showed higher determined susceptibilities for the same labeling ions. The susceptibilities determined for Er^{3+} and Ni^{2+} labeled RBCs at various concentrations are used as examples and shown in Figs. 2 and 3, respectively. Both figures show that the determined RBC susceptibilities have the same tendencies to increase or decrease with labeling-ion concentrations. The minimum number of labeling ions per RBC required for complete deposition was 1.5×10^5, as calculated by dividing 1.0×10^{-10} M labeling ions by 4.0×10^5 RBCs. All ions were assumed to be attached to RBC surfaces. The minimal magnetic susceptibility that could be determined was about 1.0×10^{-9} (cgs) by injecting Er^{3+}-labeled silicas with known susceptibilities. Susceptibilities determined with various labeling-ion concentrations were reasonably consistent with R.S.D. within 9% variation for each labeling ion and differed by 1.0–6.4% under two magnetic field intensities.

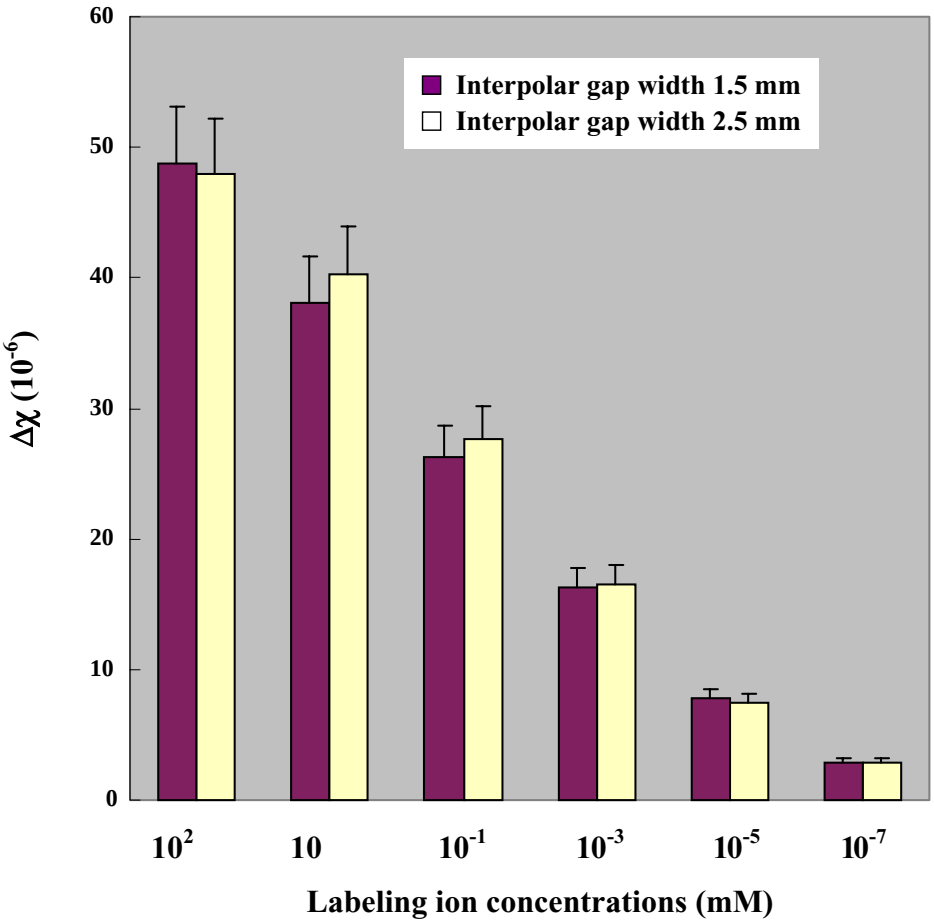

Fig. 3. Determined susceptibilities for various concentration Ni^{2+}-labeled red blood cells at two magnetic field intensities. $\nabla B^2 = (1.50 \pm 0.05) \times 10^6$ and $(5.65 \pm 0.16) \times 10^5\,G^2/\mu m$ were used for respective interpolar gap widths of 1.5 and 2.5 mm.

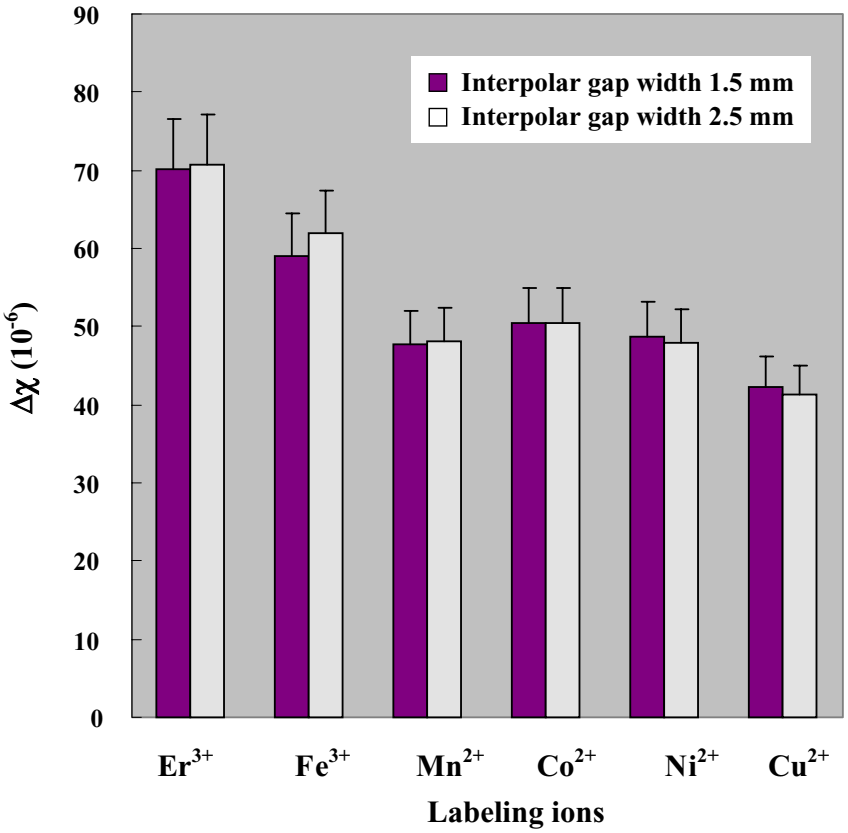

Fig. 4. Determined susceptibilities for 100 mM various ion-labeled red blood cells at two magnetic field intensities. $\nabla B^2 = (1.50 \pm 0.05) \times 10^6$ and $(5.65 \pm 0.16) \times 10^5$ G^2/μm were used for respective interpolar gap widths of 1.5 and 2.5 mm.

Determined susceptibilities using the same labeling concentrations were roughly related to their ion susceptibility magnitudes for different ions. Susceptibilities determined for various labeling ions at 100 and 10^{-3} mM labeling concentrations are shown in Figs. 4 and 5, respectively. Both figures show that Er^{3+}- and Fe^{3+}-labeled RBCs have higher susceptibilities than other ion-labeled RBC under the same labeling concentrations. The relative susceptibilities of various ion-labeled RBC determined by analytical magnetapheresis were clearly shown at 10^{-3} mM labeling

Table 2
Comparison of magnetic susceptibilities of various ion-labeled RBCs determined by analytical magnetapheresis and superconducting quantum interference device (SQUID) magnetometer

Sample	Labeling ion (10^{-5} mM)	Interpolar gap width (mm)	$\dot{V}_{max}$ (ml/min)	$\Delta\chi \pm$ S.D. (10^{-6}) (cgs) ($n = 3$) (R.S.D.)	SQUID $\Delta\chi \pm$ S.D. (10^{-6}) (cgs) ($n = 10$) (R.S.D.)
RBCs[a,b]	Er^{3+}	1.5	0.147 ± 0.001	15.7 ± 1.4 (8.9)	14.98 ± 0.52 (3.5)
		2.5	0.093 ± 0.001	15.8 ± 1.4 (8.9)	
	Fe^{3+}	1.5	0.113 ± 0.001	12.0 ± 1.1 (9.2)	12.42 ± 0.45 (3.6)
		2.5	0.067 ± 0.001	11.4 ± 1.0 (8.8)	
	Mn^{2+}	1.5	0.087 ± 0.001	9.28 ± 0.85 (9.2)	9.56 ± 0.26 (2.7)
		2.5	0.053 ± 0.001	9.00 ± 0.82 (9.1)	
	Co^{2+}	1.5	0.077 ± 0.001	8.21 ± 0.76 (9.2)	8.48 ± 0.34 (4.0)
		2.5	0.047 ± 0.001	7.98 ± 0.73 (9.1)	
	Ni^{2+}	1.5	0.073 ± 0.001	7.79 ± 0.72 (9.2)	7.26 ± 0.23 (3.2)
		2.5	0.044 ± 0.001	7.48 ± 0.69 (9.2)	
	Cu^{2+}	1.5	0.037 ± 0.001	3.95 ± 0.38 (9.6)	4.25 ± 0.16 (3.8)
		2.5	0.023 ± 0.001	3.91 ± 0.39 (10)	

The values in parentheses are in percent.
[a] Interpolar gap width = 1.5 mm, $\nabla B^2 = (1.50 \pm 0.05) \times 10^6$ G^2/μm.
[b] Interpolar gap width = 2.5 mm, $\nabla B^2 = (5.65 \pm 0.16) \times 10^5$ G^2/μm.

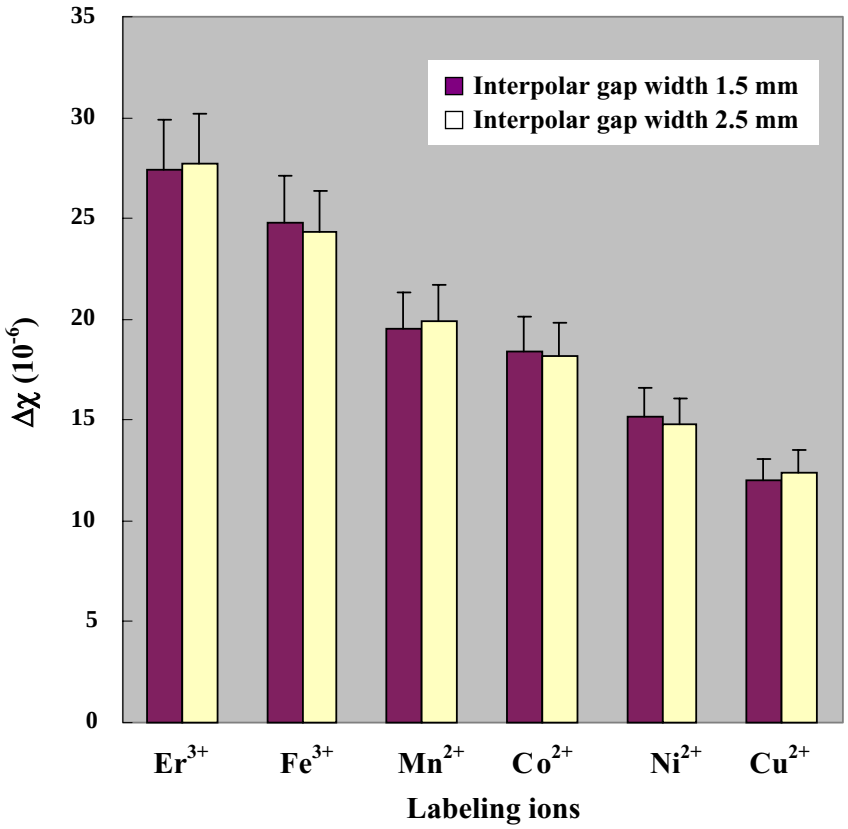

Fig. 5. Determined susceptibilities for 10^{-3} mM various ion-labeled red blood cells at two magnetic field intensities. $\nabla B^2 = (1.50 \pm 0.05) \times 10^6$ and $(5.65 \pm 0.16) \times 10^5$ G^2/μm were used for respective interpolar gap widths of 1.5 and 2.5 mm.

concentrations in Fig. 5. The relative determined susceptibilities at high labeling concentrations in Fig. 4 were not so clear as those at low labeling concentrations in Fig. 5. The number of labeling ions might be close to saturation at high labeling concentrations in Fig. 4. Table 2 shows the comparison of magnetic susceptibilities of various ion-labeled RBCs determined by analytical magnetapheresis and the reference SQUID measurement. The determined susceptibilities of various ion-labeled RBCs from analytical magnetapheresis varied 1.0–5.3% under two magnetic field intensities and were consistent with R.S.D. within 10% variation at two labeling concentrations. The susceptibilities of various ion-labeled RBCs as determined by analytical magnetapheresis were consistent with those of the reference SQUID measurements. Experimental susceptibilities determined by analytical magnetapheresis were within 10% variation and differed by 2.9–8.0% from the reference SQUID measurements.

We tried a new approach on magnetically induced particle velocity using the balance of magnetic and drag forces for particles. This new approach on magnetically induced particle velocity was successfully illustrated for determining magnetic susceptibilities of various ion-labeled RBCs in analytical magnetapheresis. The precision of each measurement was generally within 10% variation. Determined susceptibilities of various ion-labeled RBCs varied 1.0–6.4% under two magnetic field intensities. Determined susceptibilities generally differed by less than 9% from the reference SQUID measurement for high- and low-susceptibility samples. The average viabilities of these labeled RBCs after analytical magnetapheresis were 96.1 ± 0.8%. It generally took less than 10 min for susceptibility determination. Analytical magnetapheresis shows good potential for a simple, fast, and economical way to determine particle susceptibilities.

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