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Particle magnetic susceptibility determination using analytical split-flow thin fractionation

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Abstract

Magnetic split-flow thin (SPLITT) fractionation is a newly developed SPLITT fractionation subtechnique for separating magnetically susceptible particles. Here, we describe determination of particle magnetic susceptibilities using a novel method, analytical magnetic SPLITT fractionation. This method is based on particle retrieval calculation under known flow-rates and magnetic field strengths. The flow axis of separation channel was oriented parallel to gravity to exclude gravitational effects on separation. Various carrier magnetic susceptibilities, magnet interpolar gap widths, and ion-labeled particles were studied experimentally, resulting in successful determination of magnetic susceptibilities for magnetically susceptible particles and various ion-labeled particles. The minimum level of particle magnetic susceptibility (χ) that could be determined in this study was about $5.0 \cdot 10^{-7}$ (cgs). The minimum number of erbium labeling ions per particle required for silica susceptibility determination was found to be $1.57 \cdot 10^{10}$. These determined values were consistent with relative standard deviations within 8% variation and within 9% of standard reference measurements using a superconducting quantum interference device magnometer. Analytical magnetic SPLITT fractionation shows good potential for use in simple determination of particle magnetic susceptibilities.

Keywords: Split-flow thin fractionation; Magnetic separation

1. Introduction

Split-flow thin (SPLITT) fractionation describes a family of versatile techniques for separating macromolecules, colloids, and particles [1–14]. The SPLITT channel is an unpacked flow channel with a splitter at the outlet or at both inlet and outlet. A driving force or gradient is applied across a SPLITT channel perpendicular to the flow axis to selectively drive sample components into various lateral positions for separation. SPLITT fractionation (SF) may use gravitational, centrifugal, electrical, or magnetic forces for separation. Magnetic SF is a newly developed SF subtechnique for separating magnetically susceptible particles, in which perpendicular and parallel operating modes can be used for different applications [1,13,14]. In the perpendicular mode, the separation channel axis is oriented perpendicular to gravity so that gravitational and magnetic forces are applied in opposite directions to facilitate separation. In the parallel mode, the separation channel axis is oriented parallel to gravity forces so only magnetic force is used for separation, as shown in Fig. 1. We confine the present discussion to the

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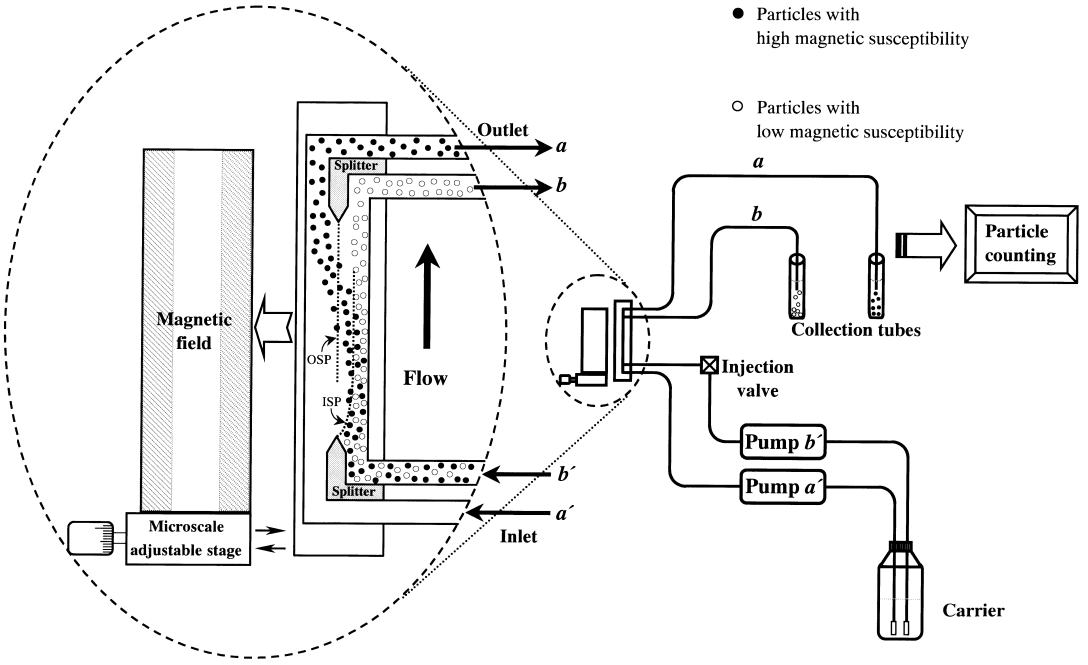

Fig. 1. General schematic of the analytical SPLITT fractionation system for particle susceptibility determination.

parallel mode for particle susceptibility determinations.

In the parallel mode of magnetic SF, samples in carriers are introduced at one inlet b′ and carriers only are introduced at the other inlet a′ at higher flow-rates to confine samples in small initial zones for better separation, as shown in Fig. 1. The imaginary boundary plane between the two inlet flows is called the inlet splitting plane (ISP) and the imaginary boundary plane between the two outlet flows is called the outlet splitting plane (OSP). The ISP and OSP are necessary to clear understanding of SF operation and their positions can be adjusted by the relative flow-rates of the inlet and outlet substreams. Magnetic force drives particles with high magnetic susceptibility (shown as solid circles) far enough toward the interpolar gap to cross the OSP and exit at outlet a. Magnetic force drives particles with low magnetic susceptibility (shown as hollow circles) less toward the interpolar gap not to cross OSP and exit at outlet b. Therefore, particles with different susceptibilities are separated into two fractions upon passage through the separation channel.

Magnetic SF like other SF family members is mainly used for preparative applications with throughputs in approximately the subgram-per-hour range, as illustrated in previous works [13,14]. Samples are continuously introduced into channels for separation in such preparative applications. However, magnetic SF, like other SF family members, can also be used to characterize samples for some applications. SF is called analytical SF when it is used for analytical applications [10,11]. Protein diffusion coefficients and particle size distributions have been determined using analytical SF. Pulsed sample injections with fixed sample loop volumes like liquid chromatography are used in analytical SF applications. Particle magnetic susceptibility is an important parameter for magnetic separation. Particle magnetic susceptibility can be used to evaluate effectiveness of magnetic separation system including magnetic labels. In this study, we characterize analytical magnetic SF for determining particle magnetic susceptibilities. Experimental variables of magnetic interpolar gap width, volumetric flow-rates, carrier magnetic susceptibilities, and magnetic ion-labeled particles that affect magnetic SF capability are investigated.

2. Theory

The theory behind fractional retrieval (Fa) calculation in the parallel mode operation of magnetic SF has been described elsewhere [14], and thus need only be summarized briefly here. Gravity plays no role in parallel mode operation since the flow axis is parallel to gravity. It therefore follows straightforwardly that Eqs. (1) and (2) below must both be satisfied to achieve complete separation:

$$bLU_{mh} \geq \dot{V}(b), \text{Fa} = 1 \quad (1)$$

$$bLU_{ml} < \dot{V}(b) - \dot{V}(b'), \text{Fa} = 0 \quad (2)$$

where b is the channel breadth, L is the channel length, $\dot{V}(b)$ is the volumetric flow-rate at outlet b, $\dot{V}(b')$ is the volumetric flow-rate at inlet b′, Fa is the fraction of particles exiting at outlet a, and U_{mh} and U_{ml} are the magnetically induced velocities of particles with high and low magnetic susceptibilities, respectively.

For particles with magnetically-induced velocities (U_m) between U_{mh} and U_{ml}, i.e., $\dot{V}(b) - \dot{V}(b') \leq bLU_m < \dot{V}(b)$, and particle fractional retrieval at outlet a (Fa) can be calculated using:

$$\text{Fa} = \frac{bLU_m - \dot{V}(b) + \dot{V}(b')}{\dot{V}(b')} \quad (3)$$

U_m can be calculated using [13]:

$$U_m = \frac{d\Delta\chi\Delta H^2}{48\eta} \quad (4)$$

where $\Delta\chi = \chi_p - \chi_c$, χ_p and χ_c are the respective magnetic susceptibilities of particles and carriers, η is the fluid viscosity, d is the spherical particle diameter or the effective spherical particle diameter, and ΔH is the drop in magnetic field strength.

We can extend Fa retrieval calculation to particle susceptibility determination by substituting Eq. (4) into Eq. (3). We can obtain the following equation after simple mathematic treatment:

$$\Delta\chi = \frac{48\eta \cdot [\text{Fa}\dot{V}(b') + \dot{V}(b) - \dot{V}(b')]}{bLd\Delta H^2} \quad (5)$$

Therefore, we can determine particle magnetic susceptibilities from known carrier magnetic susceptibilities and given remaining experimental parameters [b, L, η, d, ΔH^2, Fa, $\dot{V}(b)$, and $\dot{V}(b')$]. All these remaining experimental parameters can easily be obtained from experimental measurements.

3. Experimental

The channel length was 100 mm and the channel thickness was 0.25 mm. Channel breadths were 2.5, 5 and 10 mm for respective interpolar gap widths. The injection loop had a volume of 0.5 ml. The channels consisted of three layers of cut-out Mylar with the ends of the center piece used as inlet and outlet splitters. All layers were then sandwiched together between two channel walls of plastic sheets. Plastic screws were used beside the splitter edges to stabilize the Mylar layer.

The magnetic field was generated by a permanent magnet assembly consisting of one pair of rare-earth magnets [neodymium–iron–boron (Nd–Fe–B)] [13]. The magnets were connected by soft iron pole pieces, which conducted the magnetic fluxes to the interpolar gap. The Nd–Fe–B magnets (Super Electronics, Taipei, Taiwan) with maximum energy products of $3.0 \cdot 10^7$ G-Oe were used for all experiments in this study. Magnetic field strengths were generated using gap widths of 2, 5 and 10 mm with a gap length of 100 mm for all experiments. Magnetic field measurements were made using a Gaussmeter and a Hall effect probe (Model Gauss MG-7D, Walker Scientific, Worcester, MA, USA). The probe measured magnetic flux perpendicular to a sensing area with a diameter of 6.94 mm. Polar coordinates (r, θ) with (0, 0) located at the center of the gap were used for measurements of magnetic field strengths. Microscale adjustable stages were used to hold the magnetic probe for magnetic field measurements since magnetic fields were very sensitive near the gap center. The saturation field Bo values were 17.7, 11.2, and 6.2 kG for gap widths of 2.5, 5.0, and 10 mm, respectively. The combined magnets and pole pieces had dimensions of 175 mm × 100 mm × 6 mm and a mass of 5.5 kg. The distance between the channel and the magnetic gap was optimized for different flow-rates and accommodated particles with differing magnetic susceptibilities.

Samples and carriers were delivered into the separation channels with an LC pump (SSI series II,

State College, PA, USA) and micro tubing pumps (Eyela Mp-3, Rikakikai, Tokyo, Japan). Particle verification used light microscopy (Olympus BX-50, Tokyo, Japan). A hemacytometer (a cell counting chamber with microscalar grids having fixed volumes) was used to count particles and calculate particle concentrations. Silica particles (7.8±0.4 μm, density=2.2 g/ml) used were purchased from Hypersil (Cheshire, UK), and iron nitrate was purchased from Sigma (St. Louis, MO, USA). Erbium chloride was purchased from Strem (Newburyport, MA, USA). M-450 Dynabeads 4.5 μm in diameter (density=1.5 g/ml) were obtained from Dynal (Lake Success, NY, USA). Dynabeads are uniform, monodisperse particles doped with iron oxides. Copper (II) oxide (6.21±0.63 μm, density=6.32 g/ml), chromium (III) oxide (1.56±0.15 μm, density=5.21 g/ml), and iron (III) oxide (6.59±0.79 μm, density=5.24 g/ml) particles were purchased from Aldrich (St. Louis, MO, USA). Yeasts were purchased from a bakery shop of local market.

The carrier composition used for all particle experiments was 0.10 *M* sodium carbonate buffer with pH equal to 6.0. Opposite charge attraction between positively charged ions and negatively charged silica surfaces was used to prepare magnetic labels of silica particles. Particles and labeling ions were mixed, incubated for 60 min, and shaken every 15 min. The particles and labeling ions were centrifuged to remove the unlabeled ions after incubation. The labeled particles were then washed with carbonate buffer three times to remove residual unlabeled ions before use. Yeasts and labeling ions were also prepared using the same protocol as for silica particles.

Particle fractional retrieval (retrieval) at outlet a (Fa) was calculated using the following equation:

$$\mathrm{Fa} = \frac{\mathrm{Na}}{\mathrm{Na} + \mathrm{Nb}} \tag{6}$$

where Na is the number of particles exiting at outlet a, and Nb the number of particles exiting at outlet b. The recovery percentage of labeled particles was calculated by adding the total number of labeled particles exiting at both outlets, and dividing by the total number of labeled particles entering at the inlets. The minimum counted particles were 300 in each retrieval experiment. Sample loops of 0.5 ml were used for pulsed sample injections in all experiments.

Reference magnetic susceptibility measurements were made using an MPMS5 Model superconducting quantum interference device (SQUID) magnetometer from Quantum Design (San Diego, CA, USA). The cgs system and volume magnetic susceptibility, χ, are used throughout this study unless otherwise indicated.

4. Results and discussion

The SPLITT channel typically uses a rectangular thin channel as in this study. An annual channel used in quadrupole magnetic field was reported in the literature as a different approach for magnetic separation [15]. Briefly speaking our instrumentation in this study is much simpler than quadrupole magnetic system. This novel method measures susceptibility differences ($\Delta\chi$) between sample and carrier. Sample absolute susceptibility can be calculated from known carrier susceptibility. Sample absolute susceptibility can easily be obtained since carriers usually have negligible susceptibility versus sample. Standard magnetic particles, Dynabeads, were tested using our analytical SF method at 2, 5 and 10 mm interpolar gap widths, as shown in Table 1. Smaller interpolar gap widths would generally produce larger magnetic field strengths for specified magnets. The drop of magnetic field strength was larger for smaller interpolar gap width at each fixed total flow-rate. Dynabead susceptibilities (χ) determined at various interpolar gap widths were quite consistent with relative standard deviations (RSDs) within 6%. Table 1 shows that determined susceptibilities were also consistent with different total flow-rates at each interpolar gap width. Larger magnetic field strengths were used to balance higher total flow-rates for each interpolar gap width. The 95% confidence limit for total χ determination was 0.0198±0.0005. Determined particle susceptibilities were also consistent with RSDs within 5% at different inlet flow ratios [$\dot{V}(a')/\dot{V}(b')$] and outlet flow ratios [$\dot{V}(a)/\dot{V}(b)$] (not shown in this text). Therefore, we fixed the inlet flow ratio at 3 and outlet flow ratio at 1 for convenience during rest of our studies.

The magnetic susceptibilities of iron particles were

Table 1
Magnetic susceptibility of Dynabeads determined by magnetic SPLITT fractionation

Dynabead interpolar gap width (mm)	Inlet flow-rate (ml/min)		Outlet flow-rate (ml/min)		Fa (%) ($n=9$)	$\bar{\chi}\pm$SD (dimensionless, cgs) (10^{-6}) ($n=9$)	Total $\bar{\chi}\pm$SD (dimensionless, cgs) (10^{-6})	RSD (%)
	$\dot{V}(a')$	$\dot{V}(b')$	$\dot{V}(a)$	$\dot{V}(b)$				
2.5	[a]2.40	0.80	1.60	1.60	92.8±0.3	20400±200	20100±300	1.4
	[b]3.60	1.20	2.40	2.40	90.8±1.2	19900±100		
5.0	[c]2.40	0.80	1.60	1.60	79.2±1.4	18600±400	19500±1000	5.2
	[d]3.60	1.20	2.40	2.40	96.0±0.3	20500±300		
10.0	[e]2.40	0.80	1.60	1.60	85.2±0.6	19800±100	19900±200	1.2
	[f]3.60	1.20	2.40	2.40	93.8±0.3	19900±300		

ΔH^2 (G^2): a=540, b=817, c=325, d=485, e=133, f=208.

determined using the analytical SF method for various total flow-rates and interpolar gap widths, as shown in Table 2. Determined susceptibilities (χ) of iron particles were consistent with RSDs within 5% variation in Table 2. The 95% confidence limit of total χ determination was 0.1540±0.0033. Copper oxide (CuO) particles with relatively lower magnetic susceptibility than iron particles were also tested using the analytical SF method, as shown in Table 3. The distance between the channel and the interpolar gap used in this CuO experiment was smaller than those used for iron and Dynabeads, therefore, much higher magnetic field drops were employed for susceptibility determinations. Two lower total flow-rates were used with a 10 mm interpolar gap width due to the lower magnetic field strength than those for 2 and 5 mm interpolar gap widths. Determined particle susceptibilities were also consistent with RSDs within 6% variation, as shown in Table 3. The 95% confidence limit for total χ determination was $(2.07\pm0.05)\cdot10^{-5}$. Particle susceptibilities determined using the analytical SF method were compared with those from the standard SQUID reference method, as shown in Table 4. The mean RSDs for the analytical SF method and the SQUID reference measurement were 3.65 and 0.73%, respectively. The 95% confidence limit for total χ determination for the analytical SF and SQUID approaches were mean±0.65 SD and mean±0.44 SD, respectively. The differences between these two methods were well within 9% for particles with different susceptibilities.

The magnetic susceptibilities of iron, Dynabead, chromium oxide, and copper oxide particles were also determined in known susceptibiliy $MnSO_4$ carrier. The $MnSO_4$ solution susceptibility was $4.1\cdot10^{-5}$ as determined by SQUID measurement. Iron and Dynabead particles showed negligible particle

Table 2
Magnetic susceptibility of iron determined by magnetic SPLITT fractionation

Iron interpolar gap width (mm)	Inlet flow-rate (ml/min)		Outlet flow-rate (ml/min)		Fa (%) ($n=9$)	$\bar{\chi}\pm$SD (dimensionless, cgs) (10^{-6}) ($n=9$)	Total $\bar{\chi}\pm$SD (dimensionless, cgs) (10^{-6})	RSD (%)
	$\dot{V}(a')$	$\dot{V}(b')$	$\dot{V}(a)$	$\dot{V}(b)$				
2.5	[a]2.40	0.80	1.60	1.60	49.4±1.4	153000±4000	154000±4000	2.4
	[b]3.60	1.20	2.40	2.40	45.2±1.1	156000±4000		
5.0	[c]2.40	0.80	1.60	1.60	45.0±0.2	148000±6000	152000±6000	4.1
	[d]3.60	1.20	2.40	2.40	58.0±1.9	156000±4000		
10.0	[e]2.40	0.80	1.60	1.60	50.2±0.9	158000±2000	155000±5000	3.3
	[f]3.60	1.20	2.40	2.40	45.6±0.5	152000±6000		

ΔH^2 (G^2): a=67, b=95, c=40, d=61, e=17, f=25.

Table 3
Magnetic susceptibility of CuO determined by magnetic SPLITT fractionation

CuO interpolar gap width (mm)	Inlet flow-rate (ml/min)		Outlet flow-rate (ml/min)		Fa (%) ($n=9$)	$\bar{\chi}\pm$SD (dimensionless, cgs) (10^{-6}) ($n=9$)	Total $\bar{\chi}\pm$SD (dimensionless, cgs) (10^{-6})	RSD (%)
	$\dot{V}$(a′)	$\dot{V}$(b′)	$\dot{V}$(a)	$\dot{V}$(b)				
2.5	[a]2.40	0.80	1.60	1.60	30.9±0.2	19.8±0.6	20.1±0.7	3.5
	[b]3.60	1.20	2.40	2.40	39.7±0.5	20.3±0.7		
5.0	[c]2.40	0.80	1.60	1.60	53.2±1.0	21.6±0.7	21.2±0.8	3.6
	[d]3.60	1.20	2.40	2.40	37.7±1.1	20.7±0.7		
10.0	[e]0.90	0.30	0.60	0.60	42.0±0.8	20.7±0.2	21.0±0.4	1.6
	[f]1.20	0.40	0.80	0.80	21.7±0.6	21.2±0.3		

ΔH^2 (G^2): a=277038, b=425975, c=174851, d=244886, e=26608, f=30018.

susceptibility changes in the $MnSO_4$ carrier due to their much greater susceptibilities versus that of the $MnSO_4$ carrier. Decreased susceptibilities of chromium oxide particle in the $MnSO_4$ carrier were found to be $3.7\cdot10^{-5}$, or within the 10% difference range from measured SQUID value. Particle susceptibilities of copper oxide could not be determined in the $MnSO_4$ carrier due to its net negative susceptibility. This experiment using different carrier susceptibilities further supports the validity of the analytical SF method for particle susceptibility determination.

The particle susceptibilities of various ion-labeled yeasts were determined using the analytical SF method, as shown in Fig. 2. Yeasts were labeled with various 0.1 m*M* ions under the same experimental conditions. The labeled yeasts had decreasing susceptibilities in order from Er^{3+}, Fe^{3+}, Cu^{2+}, Mn^{2+}, to Ni^{2+}. Determined susceptibilities (χ) were quite consistent at various interpolar gap widths for each labeling ion. The mean RSD for total χ determination was 4.22%. The 95% confidence limit for total χ determination was mean±0.65 SD for each labeling ion. The particle susceptibilities of various ion-labeled silicas were also determined using the analytical SF method, as shown in Fig. 3. Silicas were labeled with various 0.01 m*M* ions under the same experimental conditions. Determined susceptibilities (χ) showed the same trend of decreasing susceptibility order for various labeling ions as the labeled yeasts. Determined susceptibilities were also consistent with most RSDs within 7% variation for various interpolar gap widths. The mean RSD for total χ determination was 6.30%. The 95% confidence limit for total χ determination was mean±0.65 SD for each labeling ion. The minimum susceptibility that could be determined using the analytical SF method was about $5.0\cdot10^{-7}$ cgs in this study. The minimum number of erbium labeling ions per silica particle required for susceptibility determination was found to be $1.57\cdot10^{10}$, as calculated by dividing $2.6\cdot10^{-7}$ *M* labeling ions by $1.0\cdot10^{7}$ particles. All

Table 4
Comparison of particle susceptibilities determined by SPLITT fractionation (SF) and superconducting quantum interference device (SQUID) magnetometer

Particle	SPLITT fractionation ($n=9$)		SQUID ($n=20$)	
	$\chi\pm$SD (dimensionless, cgs) (10^{-6})	RSD (%)	$\chi\pm$SD (dimensionless, cgs) (10^{-6})	RSD (%)
Fe	154000±5000	3.3	145600±100	0.06
Dynabead	19800±700	3.5	20000±100	0.50
Cr_2O_3	152±6	4	140±2	1.4
CuO	20.7±0.8	4	22.0±0.2	0.9

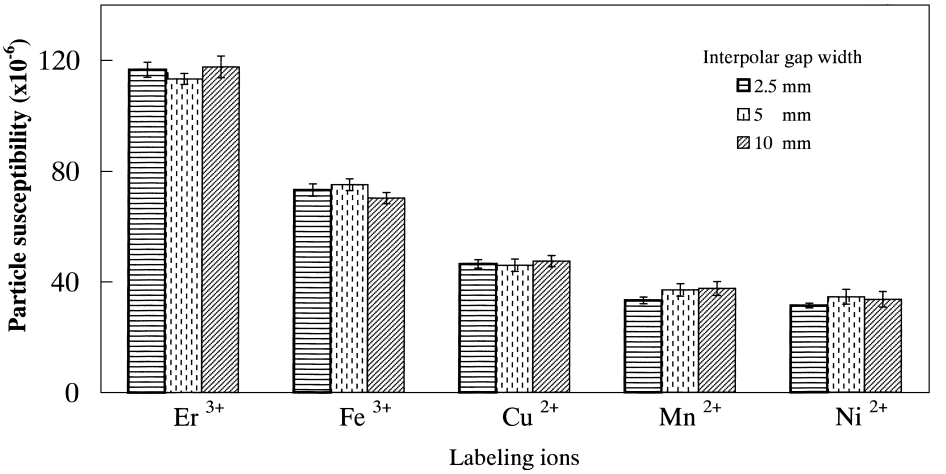

Fig. 2. Determined particle susceptibilities of various ion-labeled yeasts at different interpolar gap widths; labeling ion concentration was 0.1 m*M*. Flow-rate conditions were $\dot{V}$ (ml/min): a′ = 1.20, b′ = 0.40, a = 0.80, b = 0.80.

labeling ions were assumed to be attached to particle surfaces.

With simple theoretical treatment, analytical SF can be used for simple particle susceptibility determinations as this study shows. Experimental run times were on the order of several minutes. This method is simple and economical compared with standard reference methods, such as SQUID measurement. The determined susceptibilities of various magnetic particles and labeled particles were quite consistent with most RSDs within 8% variation and 9% difference from SQUID measurements. Recoveries in all experiments were equal to or greater than 97%. This method of particle susceptibility determination will certainly not compete with SQUID measurement in terms of accuracy, precision, measurement ranges, and field strengths. However, this method requires much simpler instrumentation and entails much lower running costs than the SQUID approach. This method promises to provide a

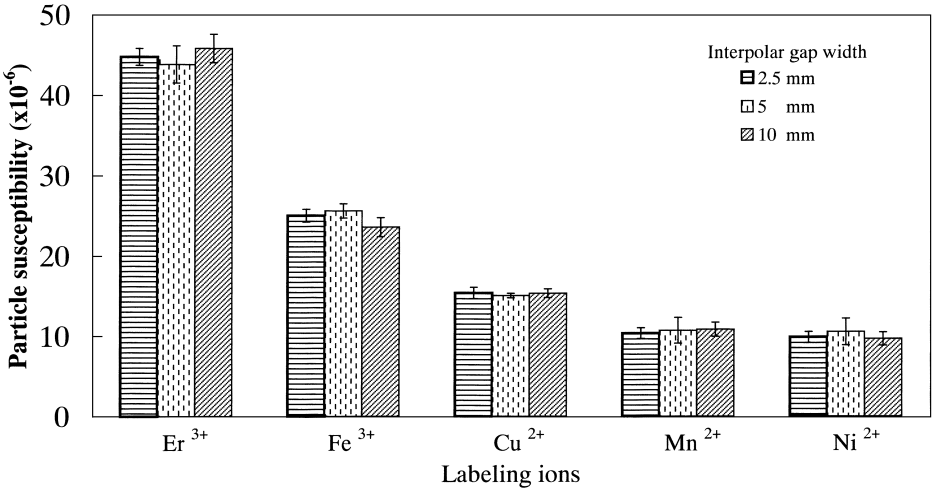

Fig. 3. Determined particle susceptibilities of various ion-labeled silicas at different interpolar gap widths; labeling ion concentration was 0.01 m*M*. Flow-rate conditions were $\dot{V}$ (ml/min): a′ = 1.20, b′ = 0.40, a = 0.80, b = 0.80.

simple, rapid, and economical method for determining particle susceptibilities.

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