Magnetophoretic Velocimetry of Manganese(II) in a Single Microdroplet in a Flow System under a High Gradient Magnetic Field Generated with a Superconducting Magnet

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An experimental system for magnetophoretic velocimetry, which could determine the volume magnetic susceptibility of a single particle dispersed in a liquid phase from a magnetophoretic velocity, has been developed. A micrometer-sized high-gradient magnetic field could be generated in a capillary by a pair of iron pole pieces in a superconducting magnet (10 T). The magnetophoretic behavior of a single particle in a capillary flow system was investigated under the inhomogeneous magnetic field. From the magnetophoretic velocity of a polystyrene latex particle dispersed in a $MnCl_2$ aqueous solution, the product of the magnetic flux density and the gradient, $B(dB/dx)$, was determined as a function of the position along the capillary. The maximum value of $B(dB/dx)$ was 4.7×10^4 T^2 m^{-1}, which was ~100 times higher than that obtained by two Nd−Fe−B permanent magnets (0.4 T). Organic droplets extracting manganese(II) with 2-thenoyltrifluoroacetone and tri-*n*-octylphosphine oxide from $MnCl_2$ solution were used as test samples. The difference of the volume magnetic susceptibility between the droplet and the medium could be determined from the magnetophoretic velocity. This method allowed us to continuously measure a volume magnetic susceptibility of 10^{-6} level for a picoliter droplet and to determine manganese(II) in the single droplet at the attomole level.

An analytical technique for micrometer-sized particles dispersed in liquid phase is greatly needed, not only in colloidal chemistry but also in environmental chemistry and biological technology.[1,2] Migration analyses have been extensively utilized to separate and characterize various kind of microparticles. Electrophoresis and ultracentrifugation are widely used as effective separation methods of biomolecules and cell composites.[3,4] The former method allows the characterization of a particle by an electrical property, and the latter can determine the density of a particle.

In our laboratory, some novel migration analytical techniques of microparticles have been developed that employ photoradiation pressure (laser photophoresis),[5] a nonuniform electrical field (dielectrophoresis),[6] or an electric current perpendicular to a homogeneous magnetic field (electoromagnetophoresis)[7] as a separation force.

A separation method utilizing a magnetic field has recently been developed, for example, a high-gradient magnetic separation (HGMS),[8] a magnetic field-flow fractionation (MgFFF),[9] and a magnetic split-flow thin fractionation.[10] These methods could separate magnetic susceptible particles; however, the migration velocities of individual partiles have not been investigated.

Recently, we demonstrated an analytical technique named magnetophoretic velocimetry, which could determine the trace amount of a magnetic compound in a single particle from its migration velocity under an inhomogeneous magnetic field.[11] The experimental setup for magnetophoretic velocimetry was simple. A pair of rare earth magnets (0.4 T), which were located so as to have a small gap of 400 μm, generated a nonuniform magnetic field. We could determine manganese(II) in an aqueous droplet dispersed in an organic solvent from the magnetophoretic velocity measured with a video microscope system. The detection limit of 6×10^{-16} mol in a single droplet was attained. However, the detectable volume magnetic susceptibility of a particle was ~10^{-4}−10^{-5}. To improve the sensitivity of the magnetophoretic velocimetry, an increase of $B(dB/dx)$ is required.

The purpose of this study is to make the technique of magnetophoretic velocimetry more sensitive by using a superconducting magnet to detect a trace amount of paramagnetic compound in a single droplet. Furthermore, a capillary flow system was employed to measure continuously the magnetophoretic effect on the flowing velocity of a particle. First, the magnetic field profile enhanced by a superconducting magnet and a pair of iron pole pieces attached to the capillary was determined from the magnetophoretic velocity of a polystyrene latex particle suspended in

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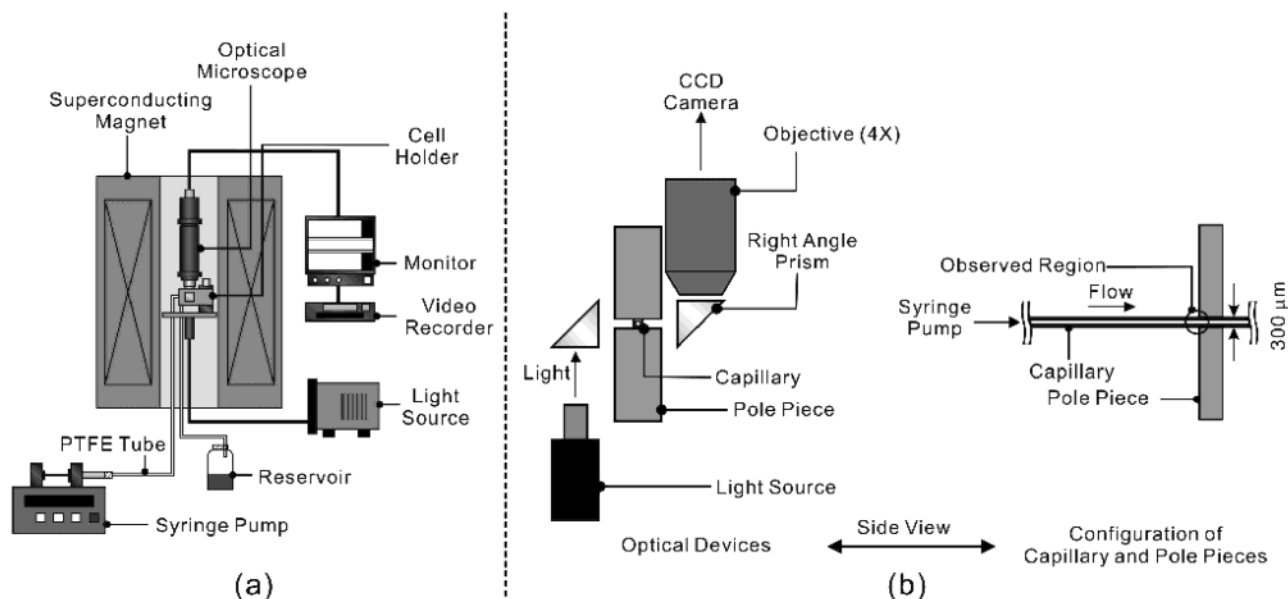(11) Suwa, M.; Watarai, H. *Anal. Chem.* **2001**, *73*, 5214−5219.

Figure 1. (a) Experimental setup for the measurement of the magnetophoretic velocity of a microparticle in liquid under a high-gradient magnetic field. The inhomogeneous magnetic field was generated with a superconducting magnet and two iron pole pieces. (b) Configuration of optical devices and pole pieces. The capillary was inserted between the pole pieces. The pole piece was $5 \times 10 \times 1$ mm, and the gap between the two pole pieces was 300 μm.

$MnCl_2$ solution.[12] Second, we observed the migration behavior of a droplet of manganese(II) chloride aqueous solution in an organic medium in order to investigate the relationship between the magnetic susceptibility of the droplet and the migration velocity under the high-gradient magnetic field. Finally, an organic droplet extracting managanese(II) dispersed in an aqueous phase was used as a test sample in which the susceptibility was varied by changing the amount of extracting reagent. The present study showed a significant effect of a superconducting magnet to improve the sensitivity of the magnetophoretic velocimetry.

EXPERIMENTAL SECTION

Polystyrene Latex Particle Dispersed in a $MnCl_2$ Solution. A suspension of the polystyrene latex particles (2.6 wt %), the diameter of which was 9.14 ± 0.71 μm, was purchased from Funakoshi (Tokyo, Japan). The polystyrene particles were dispersed in 0.05 M $MnCl_2$, 1.0 M KCl aqueous solution, the density of which was nearly equal to that of polystyrene (1.05 g cm^{-3}). The weight percentage of polystyrene particles was adjusted to 5.3×10^{-3} wt %. Manganese(II) chloride tetrahydrate and potassium chloride were purchased from Kishida Chemicals (Osaka, Japan) and Nakaraitesque (Kyoto, Japan) and were used without further purification.

Aqueous Droplets of $MnCl_2$ Solution Dispersed in an Organic Phase. A 10-μL portion of $MnCl_2$ aqueous solution was added to 5 mL of 2-fluorotoluene that had been saturated with water. Then the mixture was sonicated for ~1−3 min to disperse the aqueous solution into the 2-fluorotoluene as micrometer sized emulsion droplets. The concentration of Mn(II) was changed to the range of ~0−0.05 M. To match the densities of an organic medium and an aqueous droplet, 2-fluorotoluene (1.004 g cm^{-3}) was chosen as the organic phase. 2-Fluorotoluene (GR) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and used as received.

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Organic Droplets Containing Manganese(II) in Aqueous Phase. A 2-mL portion of a 2-fluorotoluene containing various concentrations of 2-thenoyltrifluoroacetone, TTA, and 0.05 M tri-*n*-octylphosphine oxide, TOPO, was added to a 10-mL portion of aqueous solution containing 0.01 M $MnCl_2$ and 0.01 M sodium acetate. The pH of the aqueous phase was adjusted to 4.54. The mixture in a centrifuge tube was agitated by the mechanical shaker for 3 h. After the establishment of extraction equilibrium, two phases were separated by centrifugation. A 10-μL portion of the equilibrated organic phase was added to 5 mL of the equilibrated aqueous phase and was sonicated for 5 min to prepare organic droplets with a diameter of ~4−10 μm. TTA and TOPO were purchased from Wako Pure Industries (Tokyo, Japan). These were used as received. The water was purified by a Milli-Q system (Millipore, Bedford, U.K.). The concentration of Mn(II) in the equilibrated aqueous phase was determined by an atomic absorption spectrometer (AA-6200, Shimadzu, Kyoto, Japan) to calculate the concentration in the organic phase.

Experimental Setup. Figure 1 illustrates the apparatus that was used in this study. The helium-free superconducting magnet (JMTD-10T100HH1, JMT, Japan), which had a room temperature bore of 100-mm diameter and could generate a high magnetic field up to 10 T, was used. The square glass capillary cell (Polymicro Technologies, Phoenix, AZ), which had a 100×100 μm inner section and a 300×300 μm outer section, was set between the two iron pole pieces, the sizes of which were $5 \times 10 \times 1$ mm. The capillary cell and the pole pieces were fixed to the cell holder that was positioned at the place in the bore where the magnetic field was the most homogeneous and the strongest. The capillary cell was connected with a PTFE tube to a syringe pump (pump II, Harvard Apparatus, Holliston, MA), and the sample solution was introduced at a flow rate of ~30−100 μL s^{-1}. The migration behavior of the particles around the edge of the two pole pieces was observed by an optical microscope equipped with a CCD camera (ME421, ELMO, Nagoya, Japan). The CCD image
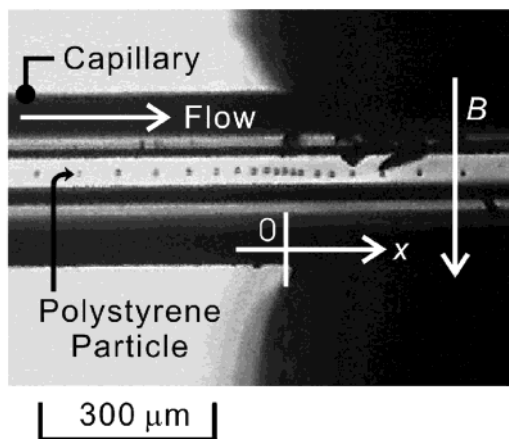
Figure 2. Example of migration behavior of a single polystyrene particle in 0.5 M MnCl₂. This micrograph was made by superimposing images that were captured at 1/60-s intervals. The diameter of the polystyrene particle was 9.14 μm.

was displayed on a monitor and also recorded on videocassettes. The migration velocity and the size of the droplet were measured from the images captured in a computer.

The magnetic susceptibility of each sample was measured by the magnetic balance (MSB-MKI, Sharewood Scientific, Cambridge, U.K.). The minimum amount required for this measurement was ∼0.2 mL. All measurements were carried out in a thermostated room at 25 ± 1 °C. The external magnetic field was set at 10 T in all experiments.

RESULTS AND DISCUSSION

Magnetophoretic Behavior of the Polystyrene Latex Particle. Figure 2 shows the typical migration behavior of a single polystyrene particle in MnCl₂ solution. This microphotograph was made by superimposing images captured in the computer at 1/60 s intervals. At the position far from the iron pole pieces, the particle moved with the constant velocity of the bulk flow. However, coming close to the edge of the pole pieces, the velocity of the particle slowed. The deceleration in the velocity indicated that there was a magnetic field gradient near the edge of the pole pieces.

Determination of Magnetic Field Profile. To determine the profile of the magnetic field produced by the pole pieces, we analyzed the velocity of the polystyrene particle, considering the force that worked on the particle in this system. We defined the edge of the pole pieces as $x = 0$ and the direction of the liquid flow as the positive direction of x as indicated in Figure 2. Under an inhomogeneous magnetic field, the total magnetic force working on a particle in the x direction, F_m, can be written as follows,

$$F_m = \frac{(\chi_p - \chi_m)}{\mu_0} V B \frac{dB}{dx} \tag{1}$$

where χ_p and χ_m are the magnetic susceptibilities of the particle and the medium, respectively, μ_0 is the vacuum magnetic permeability, V is the volume of the particle, and B is the magnetic flux density. $B(dB/dx)$ is the x component of grad$(B^2/2)$, which includes $B(dB/dx)$, $B(dB/dy)$ and $B(dB/dz)$.[11] In this system, the

y and z components are negligibly small. The magnetic susceptibility of the 0.05 M manganese(II) chloride aqueous solution, χ_m, was -0.15×10^{-6}, and that of the polystyrene latex particle, χ_p, was -8.21×10^{-6}. It was suggested that the product of the magnetic flux density and the gradient, $B(dB/dx)$, was positive near the edge of the pole pieces, because the velocity of the polystyrene particle slowed and $(\chi_p - \chi_m)$ was negative.

The magnetophoretic velocity, v_m, is defined as follows,

$$v_m = v_{obs} - v_f \tag{2}$$

where v_{obs} is the observed velocity of the particle, and v_f is the flow rate. Therefore, the magnetophoretic velocity is a relative velocity to the bulk flow rate. We assumed that in the region of approximately $x = -1000$ to -700 μm, a magnetic force did not work on the particle, and the observed velocity was equal to the bulk flow rate, v_f, because the polystyrene particle migrated with a constant velocity in this region.

Taking into account that the Reynolds number was much less than 1000 in this system, the drag force that works on a spherical particle can be expressed as

$$F_D = -6\pi\eta r v_m \tag{3}$$

where η and r are the viscosity of the medium and the radius of the particle, respectively. The magnetic force, F_m, is always commensurate with the drag force, F_D. Therefore, the magnetophoretic velocity can be written as follows:

$$v_m = \frac{2}{9} \frac{(\chi_p - \chi_m)}{\mu_0 \eta} r^2 B \frac{dB}{dx} \tag{4}$$

Since the magnetic volume susceptibilities were known in this system as shown above, $B(dB/dx)$ could be calculated using eq 4. Figure 3 shows $B(dB/dx)$ calculated from the magnetophoretic velocity of the polystyrene particles. The solid line in Figure 3 was a fitting curve obtained by Lorentz function that was expressed as

$$B\frac{dB}{dx} = \frac{h}{1 + (x - x_0)^2/w^2} \tag{5}$$

where h is the maximum value of $B(dB/dx)$ (4.71×10^4 T² m⁻¹), x_0 is the peak position (97.8 μm), and w is the half width (150 μm). This maximum value of $B(dB/dx)$ is ∼100 times larger than that obtained with the two small Nd−Fe−B magnets, as reported in the previous study.[11] This relationship between $B(dB/dx)$ and x was used in the analyses for the magnetophoretic velocimetry of manganese(II).

Magnetophoresis of a MnCl₂ Aqueous Droplet. Figure 4 is the superimposed microphotograph of magnetophoretic behavior of a single 0.04 M MnCl₂ aqueous droplet dispersed in 2-fluorotoruene. The MnCl₂ aqueous droplet was accelerated when it approached the pole pieces. The magnetophoretic velocity was increased with the concentration of manganese(II) chloride in the droplet as shown in Figure 5. The size of the droplet was in the range of ∼4−10 μm in diameter. Hence, the magnetophoretic
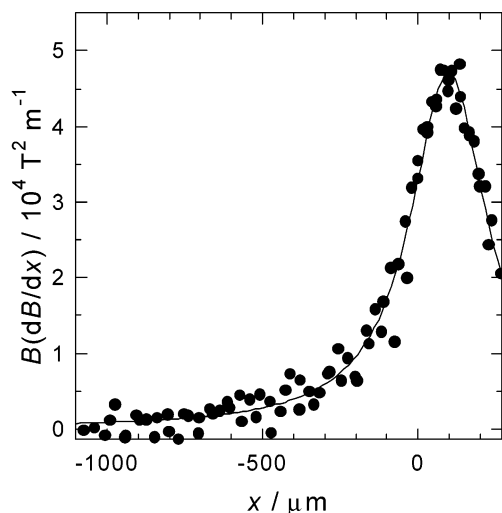
Figure 3. Experimentally determined $B(dB/dx)$ along the flowing direction, x, around the edge of the pair of pole pieces. The external magnetic flux density was set to 10 T. The solid line is the result of Lorenzian fitting eq 5. The peak height, h, was 4.71×10^4 T^2 m^{-1}; the half bandwidth, w, was 150 μm; and the peak position, x_0, was 97.8 μm from the edge of the pole pieces.
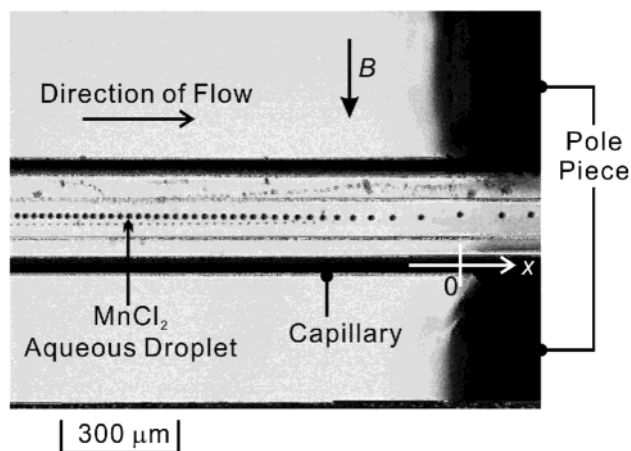

Figure 4. Typical result of migration behavior of a 2-fluorotoluene droplet extracting Mn(II) from the aqueous phase. The external magnetic field was 10 T. [TTA]$_{init}$ = 0.075 M and [Mn(II)]$_{a\ init}$ = 0.01 M.

velocities were normalized by dividing by the square of the radii in Figure 5. The magnetophoretic velocity was negative when [MnCl$_2$] = 0 M, because the magnetic susceptibilities of 2-fluorotoluene and water are -7.2×10^{-6} and -9.1×10^{-6}, respectively.

For the investigation of a relationship between a magnetophoretic velocity and $B(dB/dx)$, the magnetophoretic velocities normalized to the unit radius were plotted against $B(dB/dx)$ in Figure 6. As expressed in eq 4, the magnetophoretic velocity was directly proportional to $B(dB/dx)$ in every Mn(II) concentration, and it was possible to calculate the difference of the magnetophoretic susceptibility between the droplet and the medium from the slopes. Figure 7 shows a comparison between the magnetic susceptibilities of bulk aqueous solution measured with a magnetic balance (open circles) and those of droplets calculated from a magnetophoretic velocity (filled dots). The magnetic susceptibilities measured in both systems were in good agreement with each other and were proportional to the concentrations of MnCl$_2$.
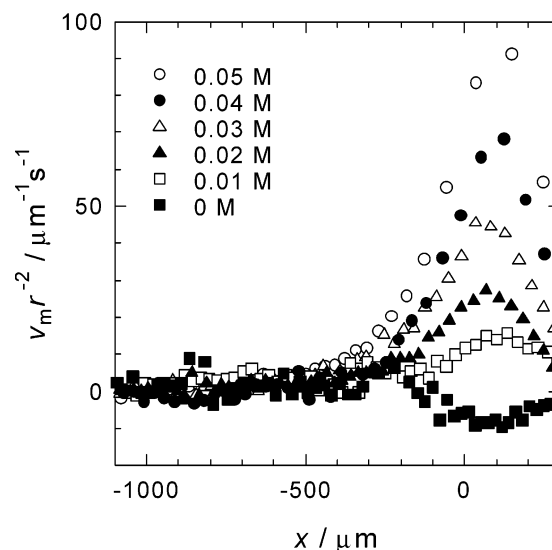
Figure 5. Magnetophoretic velocities of aqueous droplets in 2-fluorotoluene normalized by the sizes of droplets with various Mn(II) concentrations in the range of [MnCl$_2$] = ~0−0.05 M.
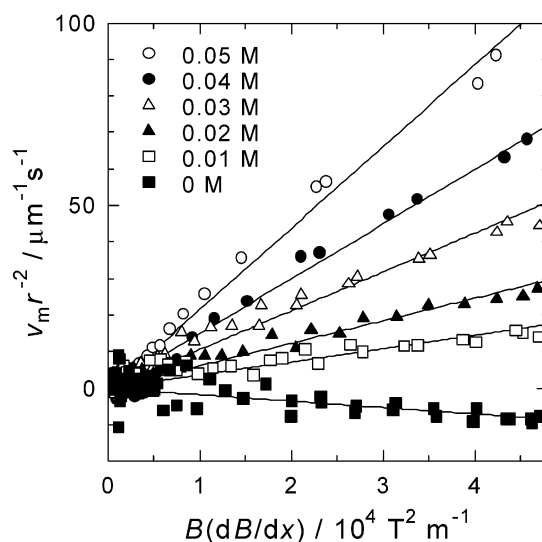

Figure 6. Normalized magnetophoretic velocities of the aqueous Mn(II) droplets in 2-fluorotoluene as a function of $B(dB/dx)$. Equation 5 was used to calculate the values of $B(dB/dx)$ at any position of x.

Hence, it was suggested that the present method could measure the magnetic susceptibility of microdroplets at the femtoliter level at a sensitivity of 10^{-6}. Moreover, the magnetophoretic velocity could determine the amount of Mn(II) in the droplet, because the magnetic susceptibility correlates with the concentration of paramagnetic species in the droplet. The detectable lower limit of manganese(II) in a single droplet was 8×10^{-17} mol from the measurable lower limit of the radius (1.5 μm) and the magnetophoretic velocity (30 μm s^{-1}). This value was 1 order smaller than that in the previous study.[11] The detection limit might be lowered 1 more order by improving the stability of the flow rate.

Magnetophoretic Velocimetry of Mn(II) Extracted Organic Droplets. The solvent extraction equilibria of Mn(II) with TTA and TOPO has been reported by Honjo and co-workers.[13] Man-

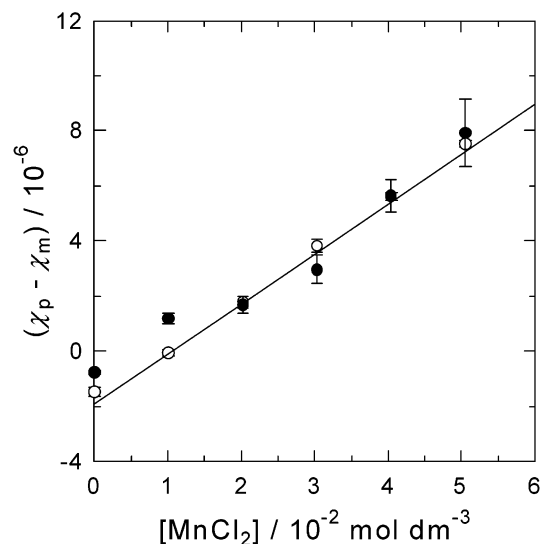(13) Honjo, T.; Horiuchi, M.; Kiba, T. *Bull. Chem. Soc. Jpn.* **1974**, *47*, 1176−1180.

Figure 7. Magnetic susceptibility of aqueous droplets as a function of the concentration of $MnCl_2$ in the droplets. The open circles were the magnetic susceptibility of the aqueous solution measured by the magnetic balance, and the solid dots were that of the aqueous microdroplet calculated from the magnetophoretic velocity. The solid line was obtained by the least-squares method of the open circles.
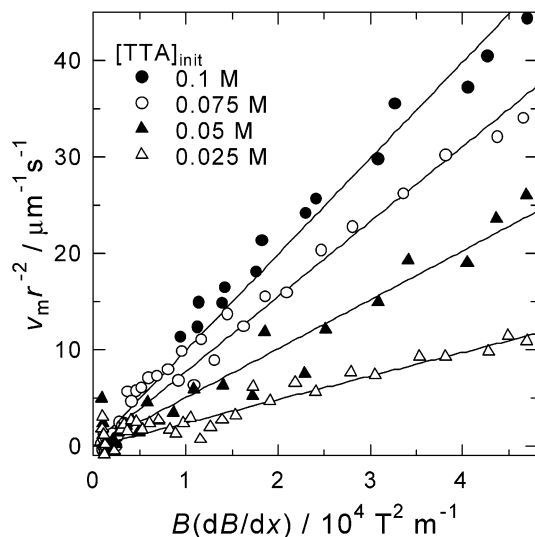

Figure 9. Comparison between the values of $(\chi_p - \chi_m)$ of the 2-fluorotoluene phases measured by the magnetic balance and those of the organic droplet calculated from the magnetophoretic velocity. The slope of the dashed line is unified. The initial concentration of TTA in the organic phase was varied in the range of ~0−0.1 M, and that of TOPO was 0.05 M.

Figure 8. Linear dependency of the normalized magnetophoretic velocities of the Mn(II)-extracting 2-fluorotoluene droplets on $B(dB/dx)$ under the various initial TTA concentrations, including 0.05 M TOPO.

Figure 10. Dependence of the magnetic susceptibilities of the Mn(II)-extracting 2-fluorotoluene droplet and the organic phase on $[Mn(II)]_o$ in the bulk organic phase.

ganese(II) was extracted into a 2-fluorotoluene phase, only when TOPO was added as a synergistic reagent.

Figure 8 shows the increase in magnetophoretic velocity of 2-fluorotoluene droplets containing TTA and TOPO with $B(dB/dx)$. The magnetophoretic velocity was normalized to the size of the droplet as well, as in Figure 6. The normalized velocity, v_m/r^2, increased linearly with the increase of $B(dB/dx)$. The difference between the magnetic volume susceptibilities of 2-fluorotoluene (-7.2×10^{-6}) and 0.01 M $MnCl_2$ aqueous solution (-7.1×10^{-6}) was very small. Therefore, the migration velocity could be changed little if Mn(II) was not extracted. The magnetophoretic velocity reflected the amount of the extracted Mn(II), the velocity increasing with the initial concentration of TTA as shown in Figure 8.
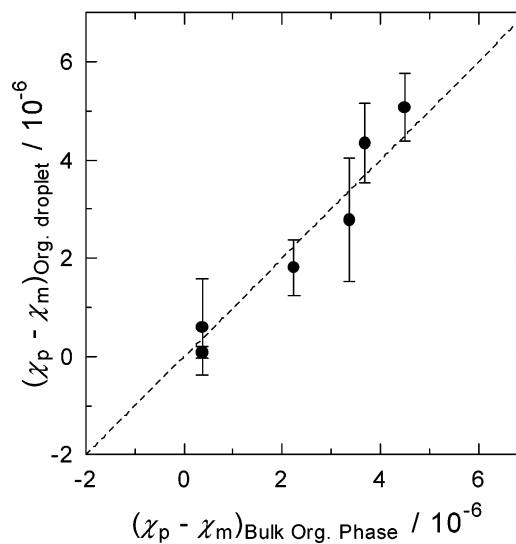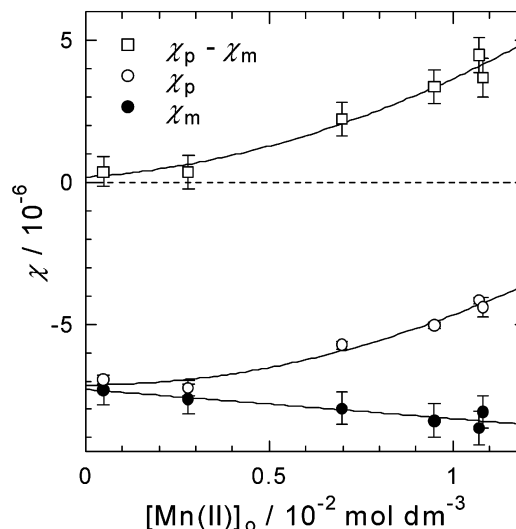
The value of $(\chi_p - \chi_m)$ calculated from the magnetophoretic velocity of the droplets was in good agreement with that obtained by the magnetic balance, as shown in Figure 9. Hence, this technique could measure the magnetic susceptibility of the Mn(II) containing microdroplets, which can hardly be done by other techniques.

Figure 10 shows the variation of the magnetic susceptibility of the organic droplets and the aqueous phase with the concentration of manganese(II) in the bulk organic phase. In contrast to the manganese(II) chloride aqueous solution system, the experimental data of the organic droplet had better correlation with a quadric function than a linear line. It might be ascribable to the probable difference of the magnetic susceptibility between $Mn(TTA)_2(TOPO)$ and $Mn(TTA)_2(TOPO)_2$, although we did not investigate this relationship further. It should be noted in Figure
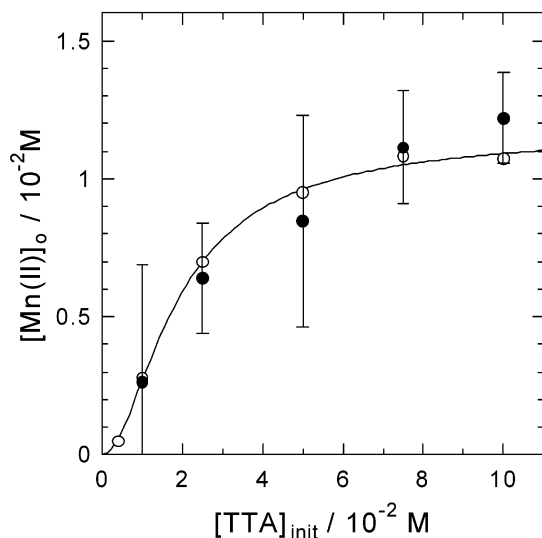
Figure 11. Manganese(II) concentrations in 2-fluorotoluene droplets calculated from the magnetophoretic velocities (solid dots). Bulk phase concentrations before dispersion (open circles) were determined by atomic absorption spectrometry.

10 that the magnetic susceptibilities of both a droplet and a medium were negative. Therefore, we could observe the magnetophoretic velocity of a diamagnetic droplet dispersed in a diamagnetic medium.

It was possible to obtain $[Mn(II)]_o$ in the microdroplet from the magnetophoretic velocity using the relationship shown in Figure 10. Figure 11 shows the agreement between the Mn(II) concentrations in the bulk organic phase determined by atomic absorption spectrometry and those in the microdroplet measured by magnetophoretic velocimetry. In the region where an initial concentration of TTA in organic phase, $[TTA]_{init}$, was larger than 6×10^{-2} M, the concentration of the Mn(II) complex in the organic phase was saturated. Hence, it was demonstrated that the magnetophoretic velocimetry could determine the concentration of manganese(II) in a single organic droplet that was at solvent extraction equilibrium state with the bulk aqueous phase.

CONCLUSION

We succeeded in generating a high-gradient magnetic field with a superconducting magnet (10 T) and a pair of iron pole pieces, and in determining the profile of $B(dB/dx)$ from the magnetophoretic velocity of a polystyrene particle. The maximum value of $B(dB/dx)$ was evaluated as 4.7×10^4 T^2 m^{-1}, which was ∼100 times larger than that attained by using two pieces of a small permanent magnet (0.4 T).

This new method will be used for a continuous magnetophoretic detection of an eluate droplet in HPLC or FIA. Using this method, it will be not necessary to separate two phases in a solvent extraction FIA, and the magnetic susceptibility of flowing droplets can be measured directly.

The notable situation of the present system is that both the droplet and the medium had diamagnetic susceptibilities (χ_p, χ_m < 0). Nevertheless, the particle showed a magnetophoretic velocity that allowed us to determine the magnetic susceptibility. In the usual magnetic separation used in industries, susceptibility is positive and at least one order larger. Therefore, the present study showed that the magnetophoretic velocimetry could allow us to characterize not only paramagnetic species but also diamagnetic compounds that have a susceptibility level of 10^{-6}. Under the high-gradient magnetic field of 4.71×10^4 T^2 m^{-1}, the particle of $r = 1$ μm and $(\chi_p - \chi_m) = 1 \times 10^{-6}$ will migrate at a velocity of ∼10 μm s^{-1}. This velocity corresponds to 10 amol of paramagnetic compound as the lowest detection limit. This will be attained by the stabilization of the flow rate of sample solution.