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New principle of electromagnetophoretic adsorption–desorption microchromatography

Yoshinori Iiguni, Hitoshi Watarai*

Department of Chemistry, Graduate School of Science, Osaka University, Toyonaka, Osaka 560-0043, Japan

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Abstract

A new principle for the chromatographic micro-separation of micrometer-sized particles in liquid has been invented by switching the electromagnetophoretic force in a capillary flow system. The principle is the combination of the Stokes force by the bulk flow and the adsorption–desorption force on a capillary inner surface controlled by an electromagnetophoretic buoyancy generated by an alternative current and a homogenous magnetic field. The observed retention profiles of test micro-particles was explained by the "zigzag" migration model mainly depended on particle size and their adsorption force to the capillary wall. By this method, we could succeed to separate polystyrene particles of 10 μm and 20 μm in diameter dispersed in 1 M KCl solution containing 0.01% Triton X-100 using only 1 mm long fused-silica capillary under 10 T.

Keywords: Electromagnetophoresis; Adsorption–desorption chromatography; Separation; Micro-particles

1. Introduction

The separation and characterization of colloidal particles in liquid phase, such as industrial particulate materials, environmental particles and living cells, becomes the principal subject in various scientific and technological fields. For the separation of macromolecules or submicrometer-sized particles, electrophoresis, size exclusion chromatography (SEC) and field flow fractionation (FFF) are currently the most popular methods [1–4]. In contrast, for separation of supermicrometer-sized particles, only several studies of FFF for separation of micro-particles have been reported [5]. Electrophoresis takes 10 h or longer for large DNA [6–8], and SEC is mainly used to separate macromolecules in the range up to 10^7 Da [9]. Sedimentation method has also poor resolution. Affinity chromatography is selective separation method for bioparticles, but is difficult to remove strongly adsorbed particles from the adsorbent. Therefore, the development of an alternative technique for the separation of micro-particles has been highly required.

In our laboratory, the applicability of various external fields for the migration of particles has been investigated and some novel analytical migration techniques of micro-particles were developed [10–13]. In this paper, we focus on electromagnetophoresis (EMP) which is migration technique using the magnetic field and the electric current as external field.

The phenomenon of EMP was studied for the first time by Kolin [14]. He proposed an idea to separate particles suspended in a conductive liquid by the electromagnetophoretic rotation combined with electrophoretic fractionation using permanent magnets [15–17]. Thereafter, the electromagnetophoretic effect has been the subject of a number of theoretical and experimental studies [18–22].

Recently, we reported the electromagnetophoretic migration velocity of micro-particles in capillary flow system using a superconducting magnet [23]. In the previous studies, we have demonstrated that the electromagnetophoretic force can be applied for the measurement of the particle–wall interaction force up to 1 nN using a superconducting magnet [24]. By this method, the adsorption force of micrometer-sized particles, such as polystyrene particles and carbon particles, in aqueous solution on a fused-silica surface was demonstrated.

* Corresponding author. Tel.: +81 6 6850 5411; fax: +81 6 6850 5411.
E-mail address: watarai@chem.sci.osaka-u.ac.jp (H. Watarai).

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The present study proposes a new principle for the separation of micro-particles in liquid, utilizing the electromagnetophoretic force in a capillary flow system. This method is based on a combination of the Stokes force by the bulk flow and the adsorption–desorption force on a capillary inner surface controlled by electromagnetophoretic force, which is actually the electromagnetophoretic buoyancy generated by the difference between Lorentz forces exerted on both particles and medium, when an electric current and a homogeneous magnetic field perpendicular to the current are applied to the system. By regulating the current under a high magnetic field, the force on the particle perpendicular to the surface of the cell has been controlled. Thus, the particles was adsorbed to or desorbed from the surface under a given flow rate. The adsorption force of particle is specific for the combination of the surface properties of particle and the wall. Therefore, by increasing the current under a flow, the particle can be detached from the wall at the specific desorption current, and separated from other particles with different desorption current. This is the first report of adsorption–desorption chromatography controlled by an electromagnetic field, which will be accepted as an innovative methodology in the chromatography family.

2. Theoretical

2.1. Principle of electromagnetophoretic adsorption–desorption microchromatography

When we apply the electric current to a conductive fluid including particles in homogeneous magnetic field perpendicular to the current, suspended particles can migrate perpendicular to both of the electric current and the homogeneous magnetic field. The electromagnetophoretic force, F_{EMP}, exerted on a particle is represented by

$$F_{EMP} = 2BV\frac{i}{S}\left(\frac{\sigma_p - \sigma_f}{2\sigma_f - \sigma_p}\right) \quad (1)$$

where B is the magnetic flux density (T), V the volume of the single particle (m), i the electric current (A), S the inner section of the cell, σ_f the electric conductivity of the fluid (S m^{-1}) [12] and σ_p the apparent electric conductivity of the particle (S m^{-1}) [13,24]. The direction of the electromagnetophoretic force was perpendicular to both the current and the magnetic force, and was opposite direction to the Lorentz force on the medium. This means that the force acting on the particle is the buoyancy from the medium. From Eq. (1) and Stokes' law, the following equations can be obtained as the electromagnetophoretic migration velocity, v_{EMP}:

$$v_{EMP} = kk'ir^2 \quad (2)$$

where

$$k = \frac{4}{9}\frac{B}{\eta S C_W} \quad \text{and} \quad k' = \left(\frac{\sigma_p - \sigma_f}{2\sigma_f - \sigma_p}\right)$$

where r is the radius of the spherical particle (m), η the fluid viscosity (Pa s) and C_W the drag coefficient of the wall [23].

The separation principle is completely different from that of chemical adsorption liquid chromatography, in which the adsorption–desorption probability is controlled by the local adsorption equilibrium. The adsorption–desorption probability in the new method is governed by the electromagnetophoretic force generated by the electric current and the magnetic field. Fig. 1a is the schematic drawing of the behavior of micro-particle in the electromagnetophoretic adsorption–desorption chromatography. By the electromagnetophoretic force, a particle in the capillary was migrated to the capillary wall and pinned against the wall. It was kept adsorbed until the reversed electric current reached to the desorption current. The increase of the current induced the desorption of the particle at the desorption current i_D as depicted in Fig. 1b. Therefore, the microparticle migrated in a zigzag manner across the flow by the electromagnetophoretic force with repeating adsorption–desorption cycle synchronized to the switching of the direction of the applied electric current as shown with dashed arrows in Fig. 1a. In this adsorption–desorption cycle, only particles, which are desorbed by the current less than i_{max}, can move ahead with the flow. Therefore, the moving distance in the direction of x-axis for one desorption–adsorption cycle, d, in the capillary with the inner diameter of w, is expressed as

$$d = \frac{w - 2r}{v_{EMP}}v_f \quad (3)$$

where v_f is the average linear velocity of the liquid flow in the capillary.

The applied current is designed as shown in Fig. 1b, in which the current is increased from 0 μA to i_{max} for the period, t_i. When the applied current was increased gradually

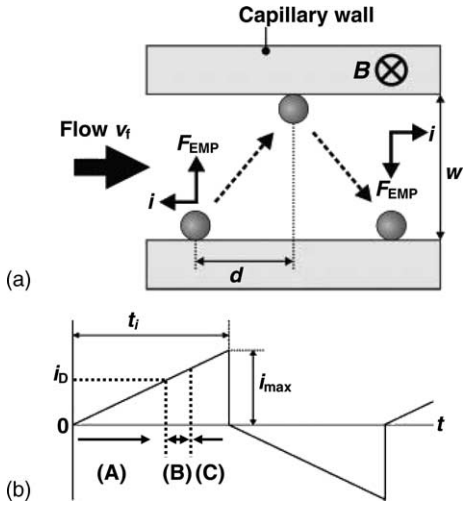

Fig. 1. (a) Schematic illustration of the electromagnetophoretic migration and adsorption–desorption of a particle in a capillary flow system, when switching the direction of the electric current. (b) Design of the applied current for the electromagnetophoresis. The current is increased from 0 μA to i_{max} for t_i, and the direction of the current is switched alternately.

(section (A) in Fig. 1b) and the current reaches to the desorption current, i_D, the electromagnetophoretic force becomes larger than the adsorption force of a particle and the particle desorbs from the capillary wall and start to migrate (section (B) in Fig. 1b). Then, the particle is pinned again to the opposite sidewall after crossing the capillary (section (C) in Fig. 1b). Finally, as the particle moves for the distance of d at every period of t_i, the retention time of the particle, t_R, in the effective capillary length, l, can be expressed as

$$t_R = \frac{l}{d}t_i = \frac{lt_i k}{v_f}\frac{k' i_D r^2}{w - 2r} \quad (4)$$

As shown in Eq. (4), the retention time is determined by k', i_D and r. Thus, micro-particles can be separated by their size and adsorption force to the capillary wall.

3. Materials and methods

To evaluate experimentally this new chromatographic principle, the separation of different sized polystyrene particles was examined. In the case of size separation of the particles made of same material, the radius and the desorption current are the predominant factors governing the retention time as shown in Eq. (4). Sample solution was 1 mol dm^{-3} KCl (Nacarai Tesque, Kyoto, Japan) solution containing polystyrene latex particles (Funakoshi, Tokyo, Japan) in diameters of 10 μm (9.14 ± 0.71 μm) and 20 μm (22.0 ± 2.7 μm), respectively, in which 0.01% (v/v) Triton X-100 (Nacarai Tesque, Kyoto, Japan) was dissolved to reduce the adsorption force of polystyrene particles to the silica surface. A fused-silica capillary (GL Science, Tokyo, Japan) and a nonionically modified fused-silica capillary whose inner diameters are 200 μm were used in our experiments. The surface was coated with squalane by the following procedure. First, a fused-silica capillary was filled with toluene solution of 3% (v/v) dimethyldichlorosilane (Shinetsu, Tokyo, Japan) and left for 5 h. After washing by methanol, water and acetone, the capillary was filled with toluene solution of 5% (v/v) squalane (Wako, Osaka, Japan) and left for 5 h, and washed by methanol and water. The capillary flow cell was made of a capillary with 2 cm in length, inserted to PTFE tubes and then covered by heat-shrink tubes. The Ag|AgCl wire electrodes, which were made by electrolysis, and peak tubes for providing sample solution were connected to the capillary cell by a three-way connector.

Fig. 2 shows the apparatus used in the present experiments. The cell was set on the holder in a homogeneous magnetic field (10 T) generated by a superconducting magnet (JMTD-10T100HH1, JMT, Kobe, Japan). Sample solutions were provided to the capillary by a syringe pump. The current was applied as shown in Fig. 1b, designed by a waveform making software (105, NF, Tokyo, Japan). A function synthesizer (WF 1915, NF, Tokyo, Japan) and an amplifier (MODEL 610-C, Trek, Tokyo, Japan) were used to provide the current to the sample solution in the capillary cell. The

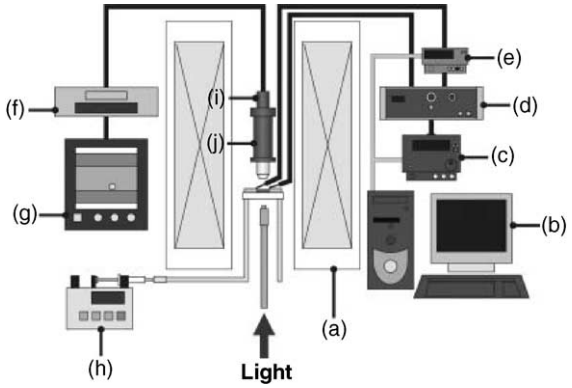

Fig. 2. Experimental set up for the adsorption–desorption chromatography using the electromagnetophoretic force: (a) a superconducting magnet; (b) PC; (c) a function syncesyser; (d) an amplifier; (e) an ammeter; (f) a video recorder; (g) a monitor; (h) a syringe pump; (i) a CCD camera and (j) a microscope.

applied current was measured by a digitalmultimeter (VOAC 7512, IWATSU, Tokyo, Japan). An optical microscope attached to a CCD camera (ME421R, ELMO, Nagoya, Japan) was used to observe the behavior of particles in the capillary. The CCD image was displayed on monitor and also recorded on a videocassette. On the monitor, the behavior of suspensions was observed and the desorption current was measured simultaneously. The time that a single particle passed through the distance of 1 mm in the capillary cell was measured as the retention time by a stopwatch. All measurements were carried out under the constant magnetic field of 10 T and the applied current period time, t_i, of 2 s in a thermostated room at 25 ± 1 °C.

4. Results and discussion

4.1. Experimental observation of retention time of polystyrene particles

Fig. 3 displays an electromagnetophoretic behavior of a polystyrene latex particle with 20 μm diameter under the flow

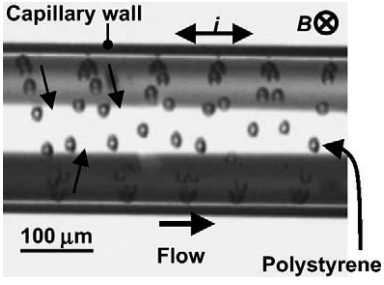

Fig. 3. Typical electromagnetophoretic adsorption–desorption behavior of a polystyrene particle in the capillary flow. The diameter of the polystyrene particle was 20 μm. A 1.0 M KCl solution was used as a conductive medium. The magnetic field was 10 T and the maximum current was 1500 μA. The flow rate was 60 μl h^{-1}. This picture was reconstructed from the images captured at a rate of 60 frame/s.

rate of 60 μl h^{-1} and the maximum current, i_{max}, of 1500 μA. The picture shown in Fig. 3 was made by superimposing images captured with every 1/30 s intervals. The particles migrated diagonally in the capillary by combination of the electromagnetophoretic force and the viscous force, and the particles, pinned by electromagnetophoretic force on the capillary wall, were not moved by the flow. It was proved that the particles just behaved in the manner predicted by the model shown in Fig. 1a.

Fig. 4 shows the retention time profiles of polystyrene particles, whose diameters were 10 μm and 20 μm, with the effective capillary length of only 1 mm. These should be called adsorption–desorption chromatogram. The maximum current, i_{max}, was 2000 μA and the flow rate was varied in the range from 60 μl h^{-1} to 100 μl h^{-1}. As shown in Fig. 4, an increase in the flow rate shortened the retention time, and the deviation of the retention time became smaller in the higher flow rate. In all flow rates, the larger polystyrene particles were retained longer in the capillary, but smaller ones eluted faster. Thus, the different sized polystyrene micro-particles could be separated. At this time, the average desorption currents were 920 μA and 1070 μA for polystyrene particles with the diameters of 20 μm and 10 μm, respectively.

In Fig. 5, the flow rate was kept at 60 μl h^{-1} and the maximum current, i_{max}, was changed at 500 μA, 1000 μA and 1500 μA. Table 1 displays the average values of the desorption current at four maximum currents. As shown in Table 1, the desorption current was dependent on the maximum current. In the lower maximum current, i.e. in the weaker pushing force, the retention time became shorter, and its deviation became smaller. This indicates that the particles pushed with stronger force are adsorbed stronger and require the larger desorption current. The calculated retention times by Eq. (4) using the desorption current of polystyrene particles with diameters of 20 μm and 10 μm are indicated in Figs. 4 and 5 as dashed lines. They are well agreement with the observed retention times and the proposed model in the present study was confirmed. The spread of the observed retention time was due to the fluctuation in the desorption current of polystyrene particles, which might be caused by the deviation in the adsorption force between the particle and the surface.

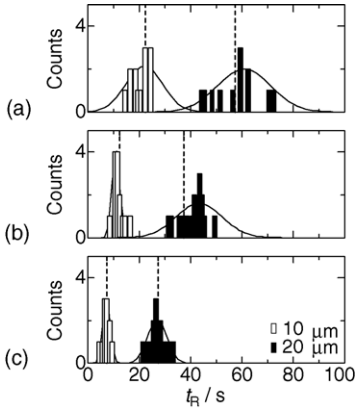

Fig. 4. Retention time of polystyrene particles whose diameters were 10 μm (white bar) and 20 μm (black bar) using a fused-silica capillary, whose inner diameter was 200 μm, at various flow rates: (a) 60 μl h^{-1}; (b) 80 μl h^{-1} and (c) 100 μl h^{-1}. Effective capillary length was 1 mm. Maximum current was 2000 μA. The dashed lines and the curves displayed that the theoretical values of retention time calculated by Eq. (4) using the observed desorption current. The lines were fitted with the Gaussian for the retention time profiles. Average values of retention time and peak width of polystyrene particles could be calculated from Gaussian fittings; $t_{R,20\,\mu m}$ = 60.9 s, 43.4 s and 27.1 s, $t_{R,10\,\mu m}$ = 22.0 s, 11.0 s and 7.19 s, $W_{20\,\mu m}$ = 41.7 s, 36.0 s and 15.1 s, $W_{10\,\mu m}$ = 28.6 s, 5.99 s and 5.80 s for 60 μl h^{-1}, 80 μl h^{-1}, 100 μl h^{-1} of flow rate, respectively.

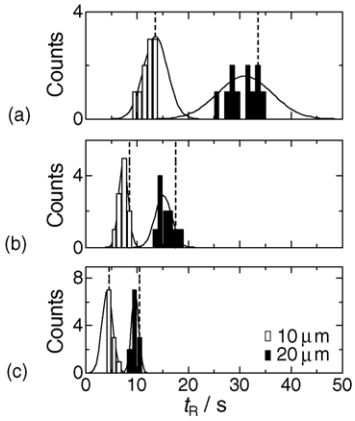

Fig. 5. Retention times of polystyrene particles whose diameters were 10 μm (white bar) and 20 μm (black bar) using a fused-silica capillary, whose inner diameter was 200 μm at various flow rates: (a) 1500 μA; (b) 1000 μA and (c) 500 μA. Effective capillary length was 1 mm. The flow rate was 60 μl h^{-1}. The dashed lines and the curves displayed that the theoretical values of the retention time calculated by Eq. (4) using the observed desorption current and the Gaussian fitting. Average values of retention time and peak width of polystyrene particles could be calculated from Gaussian fittings; $t_{R,20\,\mu m}$ = 31.0 s, 15.1 s and 9.60 s, $t_{R,10\,\mu m}$ = 13.6 s, 7.33 s and 4.21 s, $W_{20\,\mu m}$ = 21.0 s, 6.50 s and 2.77 s, $W_{10\,\mu m}$ = 9.32 s, 3.58 s and 4.18 s for 1500 μA, 1000 μA, and 500 μA of maximum current, respectively.

4.2. Resolution of size separation of polystyrene particles

Chromatographic resolution (R_s) between particles 1 and 2 was represented as

$$R_s = \frac{2(t_{R,1} - t_{R,2})}{W_1 + W_2} \quad (5)$$

where W was the width of the chromatogram peak. Tables 2 and 3 show the observed resolutions between

Table 1
The average values of desorption current of polystyrene particles from the fused-silica surface at various maximum current in the flow rate of 60 μl h^{-1}

i_{max} (μA)	i_D (10 μm) (μA)	i_D (20 μm) (μA)
2000	1070	920
1500	725	603
1000	455	312
500	253	181

Table 2
Resolution (R_s) between polystyrene particles with 10 μm and 20 μm in diameters with the effective capillary length of 1 mm at various flow rates under the applied maximum current of 2000 μA

Flow rate (μl h^{-1})	Resolution (R_s)
60	1.10
80	1.52
100	1.90

polystyrene particles with 10 μm and 20 μm in diameters calculated from the results shown in Figs. 4 and 5. The observed resolutions shown in Tables 2 and 3 were in the range from 1.10 to 1.90, which clearly indicated that these particles could be separated in the effective capillary length of only 1 mm. Moreover, better resolutions were obtained in higher flow rate; R_s = 1.10, 1.52 and 1.90 for 60 μl h^{-1}, 80 μl h^{-1} and 100 μl h^{-1}, respectively. Also, the resolutions were higher in smaller maximum current; R_s = 1.10, 1.15, 1.54 and 1.55 for i_{max} = 2000 μA, 1500 μA, 1000 μA and 500 μA, respectively, indicating that the weaker pushing force gives the better resolution. These improvement of resolutions was mainly caused by reducing the deviation of the desorption current. Thus, in the present new method, the separation performance could be well controlled by the flow rate and the maximum current.

When a squalane modified capillary was used, the observed resolution between polystyrene particles with 10 μm and 20 μm in the diameter was as low as 0.47 under the maximum current of 2000 μA and the flow rate of 60 μl h^{-1}. This value was smaller than that by a fused-silica capillary. The adsorption force of polystyrene particles on the squalane modified surface was significantly reduced in the present of Triton X-100 and was smaller than that on a fused-silica surface [24]. The particle could not be retained effectively by the squalane modified capillary. Therefore, a polystyrene particle on the squalane modified surface was flushed by the higher flow rate, because the adsorption force was weak. As found from this result, the property of the capillary inner surface has a serious influence on the resolution. Therefore, the property of a capillary inner surface was so controlled that it had a suitable adsorption force and thus a resolution in the retention time. The modification of the capillary inner surface with bio-affinity compounds will provide an additional mode in the electromagnetophoretic adsorption–desorption chromatography of bio-particles.

Table 3
Resolution (R_s) between polystyrene particles with 10 μm and 20 μm in diameters with the effective capillary length of 1 mm at various applied currents in the flow rate of 60 μl h^{-1}

Applied current (μA)	Resolution (R_s)
2000	1.10
1500	1.15
1000	1.54
500	1.55

5. Conclusion

The present study has proposed a new concept of the electromagnetophoretic adsorption–desorption chromatography for the first time. It is an innovative technique for the separation of micro-particles in a liquid by the use of the desorption–adsorption force to the capillary wall controlled by electromagnetophoretic buoyancy. Character of this method is that both adsorption and desorption have been controlled by the external physical field not by the adsorption isotherm, which is the principal mechanism in a common adsorption chromatography. Experimental results demonstrated that the proposed theoretical model was well predictable the observed retention behavior of micro-particles. The parameters used for the model calculation were determined by the observed desorption current. Furthermore, the resolution in the separation could be controlled by the flow rate and the maximum electric current. Modification of silica surface opened wide possibilities as a tool for the optimization of the resolution. Traditional methods of chromatography using a specific stationary phase such as the affinity chromatography were subject to the adsorption affinity of analyte. The advantage of the present method was that the retention profiles were optimized by the flow rate and the maximum electric current. This advantage was attained by the electromagnetophoretic adsorption–desorption cycles of micro-particles. Bio-particles' surface was so characteristic that the electromagnetophoresis adsorption–desorption microchromatography is promising for effectively characterization and separation of bio-particles according to their size and surface's property.

Acknowledgments

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