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Optimization of an Interface Chip for Coupling Capillary Electrophoresis with Thermal Lens Microscopic Detection

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This paper presents a capillary-to-microchip connection, which can be used as an interface for coupling capillary electrophoresis (CE) with a thermal lens microscope (TLM). It is difficult to directly apply TLM to samples in a capillary with a curved surface, and such an interface chip at the end of a CE separation column is needed for reliable TLM measurements. The dependence of the TLM signal intensity on the TLM detection point in the interface chip and the dependence of the theoretical plate number of CE separation on the channel dimensions of the interface chip were investigated and optimized with a mixture of 4-dimethylaminoazobenzene-4′-sulfonyl(DABSYL)-derivatized amino acids (glycine, alanine, methionine, and proline) as a model sample. By using an optimized interface chip, theoretical plate numbers of DABSYL-glycine, -methionine, -alanine, and -proline were obtained to be 104000, 95000, 104000, and 95000, respectively.

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Introduction

Capillary electrophoresis (CE) is one of the most widely used techniques for high-resolution separations of chemicals and biomolecules.[1] A wide variety of detection techniques for CE have been proposed by many researchers.[2] Each detection technique has both advantages and disadvantages. For example, a laser-induced fluorescence (LIF) is a very sensitive detection technique, but often requires chemical derivatization, because most chemical species, as they are, are not very strongly fluorescent. Although an ultraviolet-visible (UV-Vis) absorbance technique is most frequently used because of its wide applicability, it is not highly sensitive.

Thermal lens spectroscopy is a type of photothermal spectroscopy, and offers highly sensitive methods to detect the optical absorption of materials.[3] Because the most common relaxation process following the absorption of light is photothermal energy-conversion, the applicability of photothermal spectroscopy is very wide. We have developed a thermal lens microscope (TLM) that is thermal lens spectroscopy realized under a microscope.[4] With TLM, the highly sensitive detection of a wide variety of compounds in a micro space, such as in a micro chemical chip, has become possible. We have successfully demonstrated many applications of TLM to microchips.[5–7] However, TLM is hardly applicable to the detection of samples inside a capillary, because a curved surface of a capillary reduces both the sensitivity and the reproducibility of TLM.

Very recently, we developed a CE-TLM interface chip.[8] A microchip was connected to capillaries for CE separation, and was used as a window for TLM detection. An optical alignment problem caused by a curved surface of a capillary was eliminated, and TLM detection for CE was realized for the first time. Using this interface chip, we demonstrated the separation of labeled amino acids. The result showed that the detection limit determined by CE-TLM was 100-times lower than that of conventional absorbance detection. However, the performance of the analysis system was insufficient.

In this work, we examined the dependence of the TLM signal intensity on the detection point in the interface chip and the dependence of the theoretical plate number of CE separation on the channel dimensions of the interface chip, in order to optimize our interface-chip detection system and provide a detection technique for CE that satisfies both high sensitivity and wide applicability.

Experimental

Fabrication

Glass microchips were fabricated by conventional photolithography and wet-etching techniques.[9,10] A polished 0.7 mm thick Pyrex glass plate (L × W; 70 mm × 30 mm) was used as a starting material for the bottom plate. To deposit metal layers on the glass substrate, a sputtering apparatus (L-332S-

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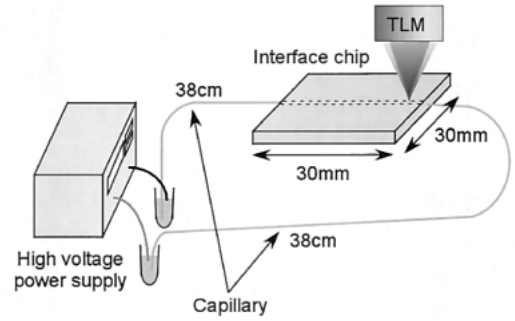

Fig. 1 Schematic illustration of the experimental setup.

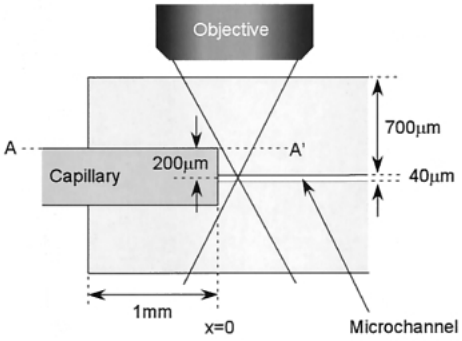

Fig. 2 Cross-sectional view of the capillary connection part of the interface chip.

FH, ANELVA Corporation, Tokyo, Japan) was used. First, 20 nm thick Cr as adhesion layer was deposited onto the substrate. Then, a 100 nm thick Au layer was deposited onto the Cr layer. A 2 μm thick positive photoresist (OFPR-800 (20Cp), Tokyo Ohka Kogyo Co., Ltd., Kawasaki, Kanagawa) was spin-coated on the Au layer and baked at 90°C for 30 min. UV light was exposed through a photomask by using a mask aligner (MA-20, MIKASA Co., Ltd., Tokyo, Japan) to transfer the microchannel pattern onto the photoresist. The photoresist was developed and a pattern with 10 μm wide lines was formed. After the Au and Cr layers were etched by I_2/NH_4I and $Ce(NH_4)_2(NO_3)_6$, respectively, and a bare glass surface with the microchannel pattern was etched with a 50% HF solution. After glass etching, the remaining photoresist and metals were removed by acetone, and by the Au- and Cr-etchants. Another Pyrex glass substrate was used as a cover plate. The cover and etched bottom plates were thermally bonded in a furnace (KDF-S70, Denken Co., Ltd., Kyoto, Japan) at 650°C for 5 h.

An interface chip comprised of a microchip and capillary tubes were prepared as follows.[8] The microchip was cut across the microchannel (100 μm width and 40 μm deep) with a dicing saw (DAD522, DISCO Corporation, Tokyo, Japan) to obtain a flat cross section of the etched microchannel. To connect the fused-silica capillary (50 μm i.d., 375 μm o.d.) at each end of the microchannel, which had a semicircular shape, an access hole was drilled so as to enlarge the microchannel cross-section. The diameter of the access holes had to match the capillary's outer diameter, 375 μm; the holes were fabricated with diamond-deposited high-speed steel drills with a diameter of 400 μm using water as a lubricant. To reduce dead volume between the capillary and the microchannel, the ends of the access holes were flattened with diamond-deposited cylindrical high-speed steel tips with a diameter of 400 μm. The capillaries were fixed to the microchip with epoxy glue.

Chemicals

4-Dimethylaminoazobenzene-4′-sulfonyl(DABSYL)-derivatized amino acids (glycine, alanine, methionine, and proline) were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and were used as-received. A concentrated stock solution of each DABSYL amino acid (1.0×10^{-5} M) was prepared by dissolving the derivatized amino acid in a pH 7.4 phosphate buffer. Sample solutions for CE separation were prepared by the stepwise dilution of a mixture of the four DABSYL amino acids stock solutions in equal quantities with pH 7.4 phosphate buffer. The sample solution was injected hydrostatically into the capillary before electrophoresis.

Measurements

Capillary electrophoresis was performed by applying a high voltage to the capillary using a high-voltage power supply (890-CE, Jasco Co., Tokyo, Japan). Capillary electrophoresis runs were monitored by using the TLM system. Figure 1 shows a schematic illustration of the experimental setup. The TLM system has been described elsewhere.[4,11] Briefly, the TLM system consisted of an optical microscope (Special design, Nikon, Tokyo, Japan), two lasers (a diode pumped Nd:YVO_4 laser (JUNO 100, 532 nm, 100 mW, Showa Optronics Co., Ltd., Tokyo, Japan) for the excitation beam and a He-Ne laser (05LHP171, 632.8 nm, 15 mW, Melles Griot, Carlsbad, CA) for the probe beam) and opto-electronic detection systems. The excitation beam, which was mechanically modulated at 1020 Hz by a light chopper (5584A, NF Corporation, Yokohama, Japan), and the probe beam were coaxially aligned by a dichroic mirror and a mirror in the bodytube of the microscope, and then introduced into an objective lens with a numerical aperture of 0.46 and 20 magnification (CF IC EPI Plan, Nikon). Transient divergence of the probe beam, induced by the periodically modulated thermal lens effect, was detected as a change in the light intensity. The probe beam intensity after passing through a condenser lens and an interference filter (03FIL006, Melles Griot) was monitored with a photodiode (ET-2030, Electro-Optics Technology, Traverse City, MI). The electrical signal from the photodiode was fed into a lock-in amplifier (LI-5640, NF Corporation). The synchronous signal with modulation frequency was acquired as a thermal lens signal using a PC with a GP-IB card (National Instruments, Austin, TX) and a custom LabView program (National Instruments).

Results and Discussion

Dependence of the TLM signal intensity on the detection point

Figure 2 shows a cross-sectional view of the capillary connection part in the interface chip. As can been seen, since the laser beams passing through the objective lens can be scattered by the capillary in the connection part, it is necessary to investigate the optimal detection point of the TLM.

Before carrying out experiments, we evaluated the effect of light scattering at the terminal edge of the capillary, which considering the geometrical arrangement of the capillary connection part, optical parameters, and refractive index of media. The intensity distribution, which has a Gaussian distribution, at a distance r ($= \tau_1\sqrt{x^2 + y^2}$) from the center of the beam is given by

$$\psi(r) = A_0 \frac{w_0}{w(z)} \exp\left[-2\frac{x^2 + y^2}{w^2(z)}\right], \quad (1)$$

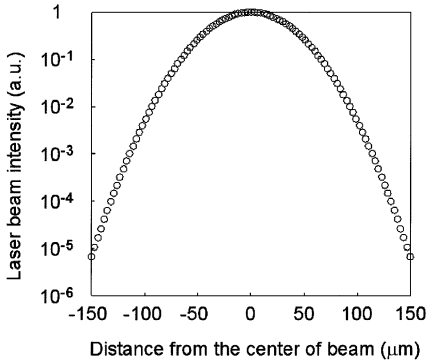

Fig. 3 Dependence of the laser intensity on the distance from the center of the laser beam.

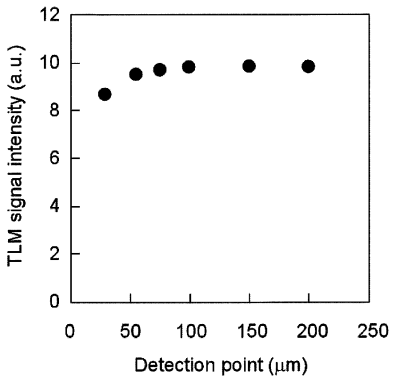

Fig. 4 Dependence of the TLM signal intensity on the detection point. Applied voltage, 18 kV; sample, 1.0×10^{-5} M DABSYL-alanine; separation length, 38 cm; sample injection, 15 s.

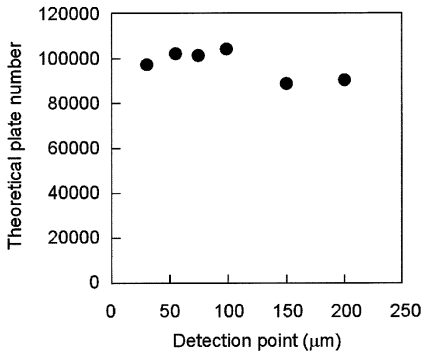

Fig. 5 Dependence of the theoretical plate number on the detection point. Applied voltage, 18 kV; sample, 1.0×10^{-5} M DABSYL-alanine; separation length, 38 cm; sample injection, 15 s.

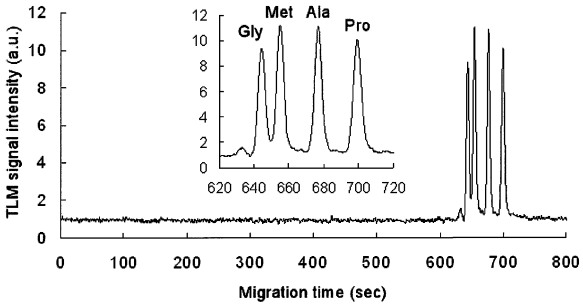

Fig. 6 Electropherogram of 1.0×10^{-5} M DABSYL-glycine (Gly), -methionine (Met), -alanine (Ala), and -proline (Pro) measured at the optimal detection point. Applied voltage, 18 kV; separation length, 38 cm; sample injection, 15 s.

where A_0 is the normalization factor, and w is the radius at which the intensity drops to $1/e^2$, and is called the spot size of the beam. For Gaussian beam propagation in free space, the spot size has the minimum value w_0 at one plane along the beam, which is known as the beam waist. The beam waist is described by

$$w_0 = \frac{\lambda}{n\pi NA'} \tag{2}$$

where n is the refractive index of glass, and NA is the numerical aperture of the objective lens. For a beam of wavelength λ at a distance z along the beam from the beam waist, the variation in the spot size is given by

$$w(z) = w_0\left[1 + \left(\frac{\lambda z}{\pi w_0^2}\right)^2\right]^{1/2}. \tag{3}$$

The intensity profile of the excitation laser in the A–A' plane in Fig. 2 was obtained by using Eqs. (1) and (3), and is depicted in Fig. 3. The origin of the horizontal axis corresponds to the position of the highest intensity, *i.e.*, the center of the laser beam. Since the light intensity outside ± 100 μm is less than 1% of the peak value, the effect of the light scattered at the capillary edge can be neglected when the center of the laser beam is positioned at a distance of more than 100 μm from the edge of the capillary.

Next, we investigated the dependence of the TLM signal intensity on the detection point. In this experiment, DABSYL-derivatized alanine was selected as a sample and their electropherograms were obtained. Figure 4 illustrates the dependence of the TLM signal intensity on the detection point. The origin of the horizontal axis corresponds to the end of the capillary ($x = 0$), as shown in Fig. 3. Although the TLM signal intensity was constant in the range of more than 100 μm away from the capillary end, the TLM signal intensity decreased at nearer points. This decrease resulted from scattering of the laser light at the edge of the capillary, as described above. This experimental result and the calculation showed good agreement.

Next, the dependence of the theoretical plate number for CE on the TLM detection point was examined using the same experimental data. The result is shown in Fig. 5. Contrary to the dependence of the TLM signal intensity, the theoretical plate number was constant within 100 μm from the junction, and became worse at more distant points. This may be explained by the influence of the junction between the capillary and the microchannel. Since the cross-sectional shapes of the capillary (circular) and microchannel (semi-circular) are not the same, disorder of the band occurred at the junction. Moreover, the electroosmotic velocity in the quartz capillary was different from that in the microchannel, which was prepared from Pyrex glass. Based on these results, we concluded that the most suitable position for our interface chip is a point 100 μm apart from the junction between the capillary and the microchannel.

Electropherograms of 1.0×10^{-5} M DABSYL-glycine, -methionine, -alanine, and -proline measured by the TLM at the optimal detection point are shown in Fig. 6. From this electropherogram, the theoretical plate numbers of DABSYL-

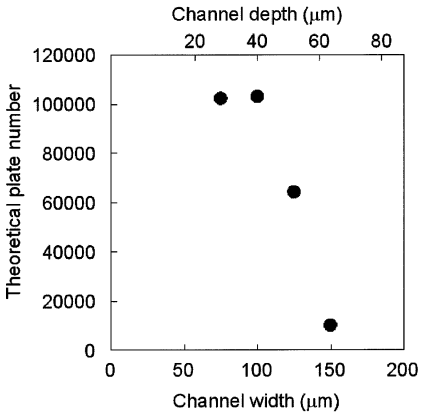

Fig. 7 Dependence of the theoretical plate number on the microchannel dimension. Applied voltage, 18 kV; sample, 1.0×10^{-5} M DABSYL-alanine; separation length, 38 cm; sample injection, 15 s.

glycine, -methionine, -alanine, and -proline were estimated to be 104000, 95000, 104000, and 95000, respectively. By optimizing the detection point, the theoretical plate number was remarkably improved from that of a previous study.[8]

Dependence of the theoretical plate number on microchannel dimension

The bands separated by electrophoresis were disturbed at the junction, as discussed above. In order to investigate the influence of the size-difference between the capillary and the microchannel, the dependence of the theoretical plate number on the microchannel dimension was measured by the TLM at a point 100 μm apart from the junction; 1.0×10^{-5} M DABSYL-alanine was used as a sample. The results are shown in Fig. 7. It is obvious that the theoretical plate number became poor above 100 μm width. A lowering of the theoretical plate number could be prevented if the microchannel with less than 100 μm width was used.

Conclusion

We investigated the dependence of the TLM signal intensity on the detection point and the dependence of the theoretical plate number on the channel dimension in order to optimize our interface chip. These optimizations lead to an improvement of the separation ability. When a mixture of DABSYL-derivatized amino acids (glycine, alanine, methionine, and proline) was employed as a model sample, the theoretical plate numbers of DABSYL-glycine, -methionine, -alanine, and -proline were obtained to be 104000, 95000, 104000, and 95000, respectively.

Another possibility for our interface chip is a combination of CE and integrated chemical processing. Since it is possible to fabricate additional microchannels on a glass substrate, on-chip postcolumn reactions can be integrated on an interface chip. A study along this line is in progress in our laboratory.

Acknowledgements

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