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Detection methods for microchip separations

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Abstract The features of analytical systems utilizing microfluidic devices, especially detection methods, are described. Electrochemical detection (EC), laser-induced fluorescence (LIF), mass spectrometry (MS), and chemical luminescence (CL) methods are covered. EC enables detection without labeling and has been used in recent years because of its low cost and sensitivity. LIF is the most generally used detection method in microchip separations. Use of LED as an excitation source for fluorescence measurement was also developed for the purpose of miniaturization of the entire system, including detection and separation. Although MS enables highly sensitive analysis, the interface between MS and micro channels is still under examination. This review with fifty-two references introduces interesting detection methods for microchip separations. Related separation methods using microfluidic devices are also discussed.

Keywords Microchannel · Electrophoresis · Detection method

Introduction

Recently, analytical systems utilizing microfluidic devices, called “micro total analysis systems” (μ -TAS) [1, 2, 3, 4, 5] has been extensively studied. They perform a series of analytical procedures, such as pretreatment including sample extraction and derivatization, separation, and detection on a small microchip. The most attractive characteristics of microchip separation arise from the size. Scientific interest in μ -TAS derives mostly from the very fast diffusion process. Diffusion effects are inversely proportional to the square of the length. When the length of a

cube is 100 μ m the mean diffusion time of small molecules is about 5–10 s. When the length of the cube is reduced to one hundredth of the original (1 μ m), mean diffusion time will decrease to sub-milliseconds. This means that reduction of the size of microchannels accelerates sample–stationary phase equilibria and, accordingly, reduces height equivalent to a theoretical plate (HETP) in a chromatographic process. Separation techniques on microchips, such as chromatography, electrophoresis, electrochromatography, and separations in immunochemical assay, have great advantages over conventional systems. The methods provide very rapid separation, versatile channel designs, very small sample volumes, and low reagent consumption. The acceleration of the molecular interaction also makes the chemical process highly sophisticated. One of the consequences of such reductions in size of the microfluidic device will be the resulting demands on the detection system. For example, the sample volume in conventional microchannel devices is less than 1 nL and the optical path length is as short as several tens of micrometers, therefore very highly sensitive detector hardware should be constructed to analyze even modest concentrations of sample solutions in the microfluidic system. Many papers have been published on microfluidic devices. We select and introduce some interesting detection methods for microchip separations from among these papers and also refer to related separation methods using microfluidic devices from our own experience.

Electrochemical detection (EC)

Although common detection schemes for microchannel electrophoresis are laser-induced fluorescence (LIF) or mass spectrometry (MS), the high cost and large size of the instrumental set-up are sometimes incompatible with the concept of μ -TAS. So the development of alternative detectors that can be micro-fabricated on a chip has attracted a great attention. Electrochemical detection (EC [6, 7, 8, 9]) has attracted great interest in recent years because of its easy operation, low cost, selectivity, and high sensitiv-

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ity without the need for additional systems usually employed by other detection methods. There is no need for the labeling demanded in LIF. Some excellent reports were found on electrochemical detection of analytes separated by a microchannel.

Fanguy and co-workers [10] developed an off-chip amperometric detector on a PDMS/glass composite microchip and applied the detector for measurement of uric acid. The nature of on-chip miniaturized electrodes limits the ability to modify the electrode surface and periodic cleaning of the surface. As applications of the microchip devices continue to expand, a surface modified electrode would be necessary for analysis of trace levels of electrically active materials. In order to develop a more versatile analytical system for the future applications they designed an off-chip disk-working electrode. Figure 1 is a schematic diagram of the microchip CE device. The chip was made of PDMS/glass composite layers and the end of the flow channel was cut into slanted cross section to enable location of the platinum working electrode just after the outlet. Using this microseparation system with off chip CE detector linear responses from 1 to 165 $\mu\text{mol L}^{-1}$ and 15 to 110 $\mu\text{mol L}^{-1}$ were found for dopamine and uric acid, respectively. The limit of detection for both compounds was about 1 $\mu\text{mol L}^{-1}$. The system was used to measure the real sample. The concentration of uric acid in a dilute urine sample could be measured in less than 30 s. The measured uric acid concentration was verified with the conventional uricase reaction method and found to be acceptable. Six additional urine samples were evaluated with the microchip device and the uric acid concentration for each sample found to be in the expected clinical concentration range. This study will be readily applicable to practical use.

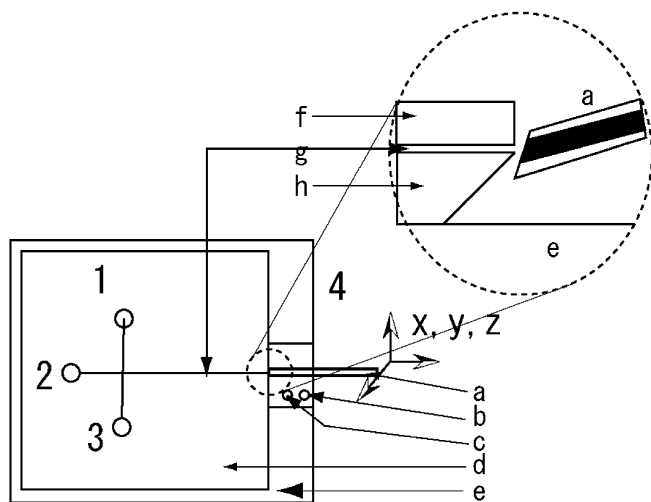


Fig. 1 Top view schematic diagram of the microchip CE device: (a) platinum working electrode clamped by x, y, z translation stage, (b) auxiliary electrode, (c) reference electrode, (d) PDMS/glass layer, (e) glass base plate, (f) PDMS layer, (g) separation channel, (h) glass layer, (1) buffer reservoir, (2) sample reservoir, (3) waste reservoir, (4) detection reservoir

On the other hand, Galloway et al. [11] developed an on-column contact conductivity detector for the analysis of various mono- and polyanionic compounds separated by electrophoresis chips fabricated in poly(methyl methacrylate) (PMMA) using hot embossing techniques. The detector consisted of a pair of Pt wires with an end-to-end spacing of approximately 20 μm (located within the microchannel). The AC waveform was applied to the electrode pair with a frequency of 5.0 kHz in order to reduce the charging current from measurement. The AC current, which is representative of solution conductivity, was recorded at the end of one pulse. Since the detector measures the conductivity of the sample solution between the electrode pair, it has general purpose use for samples with conductivity different from that of the solvent. The amino acids and peptides were separated by free-solution zone electrophoresis. A calibration plot for free alanine was found to be linear in the range 10–100 nmol L^{-1} in carrier electrolyte consisting of 10 mmol L^{-1} triethylammonium acetate. The concentration detection limit was found to be 8.0 nmol L^{-1} (corresponding to a mass of 3.4 amol in a 425 pL sample injection). The protein separations with conductivity detection were performed using MEKC. Near baseline resolution was achieved in the PMMA microchip for a solution containing eight different proteins. Also the C_{18} -modified PMMA chip with the detector could detect DNA fragments in capillary electro-chromatography mode (with a carrier electrolyte containing an ion-pairing agent). The detector is very versatile and very easy to operate. We think the detector would be readily applicable to a variety of fields in near future.

Zuborova et al. [12] applied conductivity detection to zone electrophoresis separations of proteins on a poly(methyl methacrylate) chip. Experiments were performed in the cationic separation mode (pH 2.9) with a hydrodynamically closed separation compartment and suppressed electroosmotic flow. The test proteins reached the detector in less than 10 min and their migration times showed good repeatability (0.1–0.6% RSD values). Chip-to-chip agreement of migration times was within 1.5% RSD. For conductivity detection of protein, loaded on the chip at 10–1000 $\mu\text{g mL}^{-1}$ concentrations, detection responses were characterized by 1–5% RSD values of their peak areas. They showed reproducible baseline separations of a five-constituent mixture (cytochrome *c*, avidin, conalbumin, human hemoglobin and trypsin inhibitor). On the other hand, a high sample injection channel/separation compartment volume ratio of the chip (500 nL/8500 nL) restricted resolution of proteins of very close effective mobilities, despite use of electric field stacking in the initial phase of the separation. The maximum macroconstituent/trace constituent ratio attainable for proteins on the chip was assessed for cytochrome *c* (quantifiable when its concentration in the loaded sample was 10 $\mu\text{g mL}^{-1}$) and apo-transferrin (containing a trace constituent migrating in the position of cytochrome *c* detectable when the load of apo-transferrin was 2000 $\mu\text{g mL}^{-1}$). This assessment indicated that a ratio of 1000:1 is attainable with the aid of conductivity detection on the present chip. The contact conduc-

tivity detection provided a reliable and sufficiently sensitive technique for monitoring the ZE runs with test proteins in their work. However, they point out that interactions of some proteins with the electrode surfaces cannot be excluded and disturbances attributable to these interactions can occur.

The next work is a simple and unique example of the fabrication of a microfluidic system using laser-printed polymer films [13] with a conductivity detector. Although this is not a paper on the development of electrochemical detection, we could easily understand an advantage of using electrochemical detection with polymeric microchip. A conventional laser printer was used to deposit toner selectively on a polyester film. The film was subsequently laminated against another polyester film. The toner layer binds the two polyester films and acts as a pillar to allow the blank regions to form channels for microfluidics. The channel could be easily generated by computer software. The depth of the channel, which was determined by the diameter of the toner particles, was about $\sim 6\text{ }\mu\text{m}$. Consequently, approximately twice this depth could be obtained by laminating two printed films. The resulting devices were stable when exposed for 24 h to aqueous solutions of H_3PO_4 , NaOH, methanol, acetonitrile, or sodium dodecyl sulfate. Electric tests with an impedance analyzer and microchannels filled with KCl solution demonstrated that:

1. wide channels suffer from deformation of the top and bottom walls due to the lamination of the polyester films; and
2. the toner walls were porous.

Although these drawbacks limit the maximum width of a channel and the minimum distance between two channels, the process is an attractive option to other expensive, laborious, and time-consuming methods for microchannel fabrication. The process has been used to implement devices for electro-spray tip and capillary electrophoresis with non-contact conductivity detection.

Laser induced fluorescence (LIF)

The size effect, one of the most attractive characteristics of chips, is because of rapid diffusion within small microchannels, and the effect is inversely proportional to the depth (or width) of the channel. Although the size effect enables high throughput analytical systems, the amount of sample decreases concomitantly with size, so we need highly sensitive detection methods. Among the several detection methods, laser induced fluorescence (LIF [14]) is the most widely used and will be the first choice for detection of microchip separation, despite its limitations (only applicable for fluorescent samples or samples capable of fluorescent labeling). Nevertheless, LIF has now attained its position of a standard detection method for microchip separation.

In order to perform high-throughput analysis for DNA digests, continuous analysis of DNA restriction enzyme digest on a micro-fabricated device was reported with

minimal intervention and enhanced time resolution [15]. A 62-base-pair fragment of dsDNA containing a KpnI site was used as a sample to demonstrate this process. A fused silica capillary was used in order to transfer sample solution from a single reaction mixture in the reaction vial to a micro-fabricated chip with parallel separation lanes. The 6-carboxyfluorescein-labeled DNA fragments were detected with a CCD camera as they separated in the lanes, which were filled with linear polyacrylamide for molecular sieving. The products of the restriction enzyme digest were monitored for up to 60 min at an average sampling rate of 1 injection/46 s, with consecutive injections as short as 1 injection/14 s. The digest was directly injected into the chip by means of a robot, eliminating the need for any sample-handling steps after addition of the enzyme to the reaction mixture. The effects of temperature and restriction enzyme concentration were also described. This paper shows the potential of this method to provide useful information about the process of restriction enzyme cleavage with high throughput.

Detection of DNA is not difficult because we can choose several kinds of cationic intercalating agent for the DNA strand that are fluorescent in the intercalated form but not in the free form. The dyes are simply added to the electrolyte. For protein, however, we have no such dyes. Fluorescent detection of protein usually requires labeling with an appropriate reactive fluorescent reagent. In SDS-PAGE separation of proteins, however, Lin and co-workers [16] found that use of nano-orange dye was feasible. The analysis of proteins under denaturing conditions is routinely performed with SDS-polyacrylamide gel electrophoresis. The automated capabilities of CE, use of non-gel sieving matrixes, and on-line optical detection by either ultraviolet (UV) absorption or LIF promise to revolutionize this method. Although direct on-line detection of proteins is possible as a result of their intrinsic ability to absorb UV light, LIF is a more powerful means of detection but requires pre- or postcolumn fluorescence labeling of the proteins. The development of a procedure analogous to that used for double-stranded DNA analysis, in which fluorescent intercalating dyes are simply added to the separation medium, would simplify size-based protein analysis. This would avoid the complications associated with covalent modification of the proteins but still exploit the sensitivity of LIF detection. They demonstrated that this is possible with CE and microchip detection by incorporating, in the running buffer, a fluorescent dye that interacts hydrophobically with protein-SDS complexes. The key to this is a dye that fluoresces significantly when bound to protein-SDS complexes but not when bound to SDS micelles. Comparison of electropherograms from CE-based denaturing protein analysis with UV and LIF detection indicates that the presence of the fluorescing group does not alter separation of the proteins. Moreover, comparison with electropherograms generated from microchip electrophoresis with LIF detection shows that equivalent patterns can be obtained. Despite the non-optimized nature of this separation system, a dynamic labeling procedure that enables LIF detection for proteins is at-

tractive and has the potential to circumvent the tedious labeling steps typically required.

In recent years light-emitting diodes (LED) with very high output power and shorter wavelengths have become commercially available. Because LED are not very expensive and can be driven by low power, we can even use them as a disposable light source. However, the bandwidth of LED is usually 20–30 nm (half bandwidth) which leads to difficulty filtering the LED light from fluorescence emission when the LED is used as a light source for fluorescence measurement. Chabinyc and co-workers [17] described an integrated fluorescence detection system in a PDMS microchannel with a combination of a blue LED (light source) and micro avalanche photodiode (μ -APD). The prototype micro device consisted of a re-usable detection system and a disposable microfluidic system. In the first step a microfluidic channel was fabricated in PDMS in which a multimode optical fiber (100- μ m core diameter) had been encapsulated. The tip-end of the fiber was placed adjacent to the side-wall of one of the channels. The optical fiber was used to direct light into the microchannel for excitation of fluorescent analytes. The photo-detector (μ -APD) was embedded in a slab (1 cm) of PDMS. A thin (80- μ m) colored polycarbonate filter was placed on the top of the embedded μ -APD to absorb scattered excitation light before it reached the detector. The detection face of the μ -APO was placed below the microchannel orthogonal to the tip of the optical fiber, resulting in fluorescence detection geometry. A blue LED was used for fluorescence excitation. The detection limit of fluorescein by the prototype was about 25 nmol L⁻¹ (the minimum detectable concentration of a solution of fluorescein). The device was successfully used to detect the separation of a mixture of proteins and small molecules by capillary electrophoresis.

Other literature describing the use of LED is the report by Uchiyama and co-workers [18]. They made an integrated microfluidic device in polyester. A blue LED was incorporated as a fluorescence-excitation light source into a polyester microchannel chip fabricated by in-situ polymerization. Placing the light-emitting face of the diode close to the microchannel obviated any need for an additional optical arrangement. Fluorescence from the sample was transmitted by a multi mode optical fiber incorporated into the microchip perpendicular to the LED face. FITC labeled amino acids were separated in the presence of 5 mmol L⁻¹ SDS and then were detected by LED-induced fluorescence. Miniaturization of the detection system, in particular, will be a hard obstacle to overcome. The method, based upon in-situ polymerization on an appropriate template, is expected to enable direct incorporation of components into the microchip. Figure 2 shows a close-up view of the detection region in the chip. The method will be useful for on-site separation analysis.

A microchip with both LIF and EC detection has been developed by Lapos et al. [19]. Figure 3 shows schematic representation of the microchip. Detection limits for dopamine, catechol, NBD-arginine, and NBD-phenylalanine were 448 nmol L⁻¹, 1.52 μ mol L⁻¹, 16 μ mol L⁻¹ and

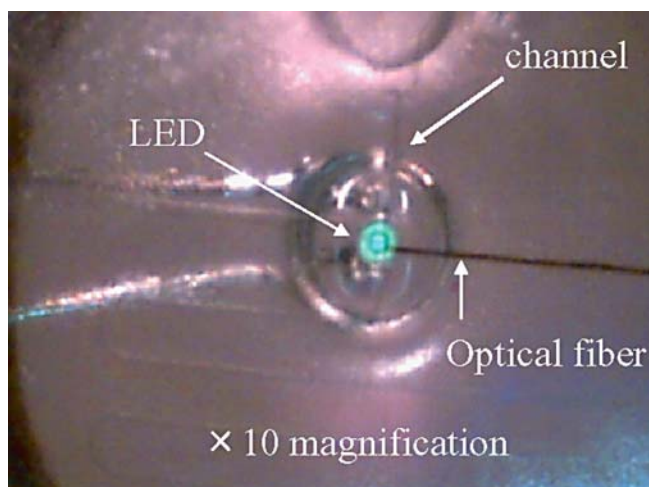


Fig. 2 Close-up view of the LED-IF detector in the chip. The face of the blue LED is placed on the microchannel. The optical fiber is placed perpendicular to the LED in the configuration for fluorescence measurement

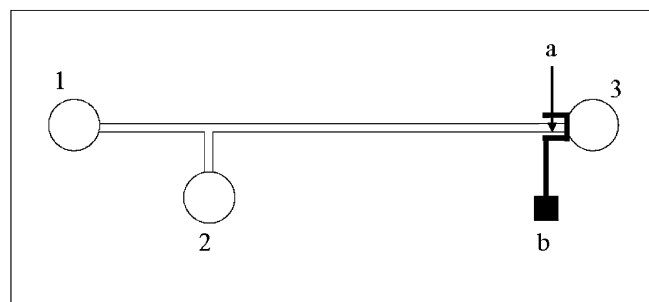


Fig. 3 Schematic representation of the functional parts of the microchip employed. The laser beam for LIF detection is placed at the channel outlet and the detection electrode is aligned $\sim 55 \mu$ m from the etched channel exit: (a) beam path, (b) working electrode, (1) buffer reservoir, (2) sample reservoir, (3) detection reservoir

28 μ mol L⁻¹, respectively. These two orthogonal detection schemes enable analysis of a wider range of compounds per separation, leading to higher throughput and enabling resolution of two neutral analytes, NBD-arginine and catechol. In addition, insight into the detection and separation mechanism is realized. Differences between migration times and peak widths for the two detectors were compared, and provided better understanding of detector alignment. A common problem encountered in electrophoresis is run-to-run migration time irreproducibility for some samples. This novel microchip dual detection system has been used to reduce this irreproducibility. An unknown sample is monitored with one detector while a standard is added to the sample and monitored simultaneously with the other detector. This dual detection method is demonstrated to normalize unknown peak mobilities in a cerebral spinal fluid sample.

Mass spectrometry

CE–MS detection using chip CE has studied extensively by many researchers [20, 21]. Increased interest in proteomics and the analysis of biological samples present at very low concentrations or, often, in limited amounts have increased demands for this sensitive and highly valuable analysis system [22]. Microchannel separations have great advantages – including rapid and highly efficient separation of very small amounts of sample. Although the principle of the MS detection by chip is the same as for conventional fused silica, use of a chip adds merits of the characteristics of the chip itself. Most chip-based devices have been made from glass [23]. One detection technique that attracts much interest is electro-spray ionization (ESI) coupled with time-of-flight mass spectrometry (TOF-MS), because of its ability to detect large bio-molecules with high sensitivity. Many attempts have been made to devise an interface between chip and ESI-MS [24, 25, 26, 27]. Almost all reports described efficient sample introduction into MS spectrometer, i.e. much effort has been devoted to establishing a well-defined Taylor cone at the exit of the microchannel where the separated sample zone emerged.

A unique application of a microfluidic chip to CE–MS determination was reported by Kameoka and co-workers [27]. A polymeric microfluidic chip made of Zeonor 1020, commonly used in the manufacture of compact disks and digital video disks, was fabricated by means of conventional embossing techniques to perform capillary electrophoresis for selected-ion monitoring and selected-reaction monitoring mass spectrometric detection of small molecules. The microfluidic channel was embossed in the polymer from a silicon master. The embossed chip was thermally bonded with a cover chip to form an enclosed channel. The channel (60- μm width, 20- μm depth, 2.0- and 3.5-cm length) enabled capillary electrophoresis (CE) separation of polar small molecules without treatment of the polymer surface. A micro-sprayer coupled via a micro liquid junction provided direct electrospray mass spectrometric detection of CE-separated components. An electric field of 0.5–2 kV cm^{-1} was applied across the microspray. Figure 4 shows the configuration of microspray and microchip. The chip was mounted on an x, y, z stage and coupled to the microspray via the micro liquid junction formed between the chip and sprayer. A separation buffer reservoir enabled separation of carnitine, acylcarnitine, and butylcarnitine with efficiencies ranging from 1650 to 18,000 plates. Injection quantities of 0.2 nmol of these small polar molecules were separated without surface treatment of the polymer. Abundant ion-current signals were observed and baseline separation of these compounds was achieved in less than 10 s. These results might accelerate the use of polymeric chip-based devices for ion spray CE–MS applications.

This work used make-up solution as a sheath flow. Although the method had good sensitivity and robustness and enabled analysis without cross-contamination, the drawback was the increased dead volume, with subsequent disadvantages for real applications.

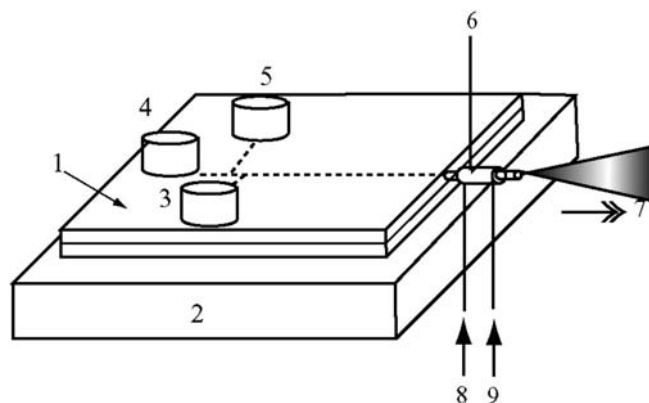


Fig. 4 Configuration of microspray and microfluid chip. The chip was mounted on an x, y, z stage positioner and adjusted to form an optimized microliquid junction: 1. bonded microchip, 2. x, y, z stage, 3. sample, 4. buffer reservoir, 5. waste, 6. +3000 V, 7. to MS, 8. make-up solution, 9. nebulizing gas

To overcome these problems Svedberg and co-workers [28] developed a sheathless electrospray constructed from polymeric microchips with conducting layer on the emitter tip. The electrospray emitter tips were fabricated directly from the end of the microchips that were made of polycarbonate or poly(methyl methacrylate) with injection molding. A variety of tip shapes and conducting coatings were evaluated using an electrospray TOF-MS run in the sheathless mode. Stable electrospray was obtained both from hand-polished and machine-milled three-dimensional tips coated with either polymer-embedded gold particles or graphite particles as the conducting layer. The relative standard deviations of the response received from the ion current of the MS analysis were in the range 3.5–12%. The detection limit for a standard mixture of five neuro-peptides was lower than 0.5 fmol. The low detection limit makes the emitter tips highly attractive for the analysis of low-abundance biological species.

Tachibana et al. [29] described a robust and simple interface for microchip electrophoresis–MS. A spray nozzle was connected to the exit of the separation channel of the microchip by use of a polyether ether ketone screw without glue, allowing easy replacement. ESI voltage was applied through a liquid junction, because the conductive coating on the spray nozzle had an extremely short lifetime. Liquid flow and sample injection were performed solely by control of the applied voltage. Using this instrumental set-up a few basic drugs were separated by chip electrophoresis then direct ESI sample injection. They found that the separation efficiency was improved by using a high-viscosity separation buffer and spray nozzle with a small bore size (20 μm), probably because of a change in the flow profile in the spray nozzle. The system was then successfully applied to the separation of peptides and protein–trypsin digests.

Chemical luminescence method (CL)

Many advantages of CL detection in conventional CE have been reported [30, 31, 32]. The method can achieve highly sensitive CE detection at the single-molecule level [33] and has a wide dynamic range of responding signal intensity. The most attractive characteristic is the simple instrumental set-up, i.e. there is no need for a light source, although the method requires optimization of the interface with the CL system for the specific sample separated.

Liu and Terabe [34] reported a CL detection system integrated with microchip capillary electrophoresis fabricated in PDMS. Two model CL systems were involved – the metal-ion-catalyzed luminol-peroxide reaction and the dansyl species conjugated peroxalate-peroxide reaction. Different strategies based on three chip patterns (cross, cross combining with Y, and cross combining with V) to perform on-line CL detection were evaluated and compared in terms of sensitivity, reproducibility, and peak symmetry. The chip pattern of cross combining with Y proved to be promising for the luminol-peroxide CL system whereas the chip pattern of cross combining with V was preferred for the peroxalate-peroxide system. A detection limit down to sub-micro molar concentrations was achieved. Successful separation of three metal cations such as Cr(III), Co(II), and Cu(II) and chiral recognition of dansyl phenylalanine enantiomers were accomplished within 1 min.

Although the next discussion is not about microchip electrophoretic studies, many useful results for CL detection are shown. Marquette and Blaum [35] reported self-contained reactant biochips for electro-chemiluminescent determination [36, 37, 38] of glucose, lactate and choline. The sensing layers were composed of enzyme-bound beads co-entrapped in a photopolymer with luminol charged beads. The layer was formed to spot them at the surface of a glassy carbon electrode. After optimization of the applied potential, the integration time and the luminol charge in the sensing layer, the electro-chemiluminescent H_2O_2 sensor had a detection limit of $2\text{ }\mu\text{mol L}^{-1}$. The sensor detects glucose, lactate, and choline in the ranges $20\text{--}20,000\text{ }\mu\text{mol L}^{-1}$, $2\text{--}200\text{ }\mu\text{mol L}^{-1}$ and $2\text{--}200\text{ }\mu\text{mol L}^{-1}$, respectively.

Yakovleva and co-workers [39] also describe a microfluidic enzyme immunosensor with immobilized protein A and G which uses chemiluminescence detection. Affinity proteins were covalently immobilized on silicon microchips comprising 42 porous flow channels $235\text{ }\mu\text{m}$ deep and $25\text{ }\mu\text{m}$ wide. The chip was used to develop microfluidic immunosensors based on HRP, catalyzing the chemiluminescent oxidation of luminol with *p*-iodophenol. The silica surfaces were chemically modified to attach protein A or G. The chip itself is multi-parametric for glucose, lactate, and choline. The protein G-based immunosensors were regenerated with 0.4 mol L^{-1} glycine-HCl buffer, pH 2.2, with no loss of activity observed for a storage and operating period of over 8 months. The immunosensor was applied to the analysis of real samples;

protein G immobilized microchips were used to detect atrazine in surface water and fruit juice spiked with known amounts of the atrazine. The recoveries were 87–102 and 88–124% at atrazine fortification levels of $0.5\text{--}3$ and $80\text{--}240\text{ }\mu\text{g L}^{-1}$, respectively.

Other detection method

New proposals for detection on microchips were found that do not belong to any category above. Although UV absorption measurement is not very sensitive among the detection methods used, it is the most widely used in HPLC and conventional CE, because of its convenience and stability and the range of samples detected. There are, however, few instruments commercially available for absorption measurements in chip experiment, researchers should prepare the absorption measuring system because of the flat shape of the microchip and optical geometry of the instrument.

Lu et al. [40] developed a microscopic absorption measurement system utilizing green LED as a light source [41] and applied it to the separation and detection of trinitroaromatic explosives. A CE microchip was applied to the separation and detection of three trinitroaromatic explosives, 1,3,5-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), and 2,4,6-trinitrophenyl-*N*-methylnitramine (tetryl), in the presence of ten other explosives and explosive derivatives in non-aqueous electrolyte (acetonitrile-methanol 87.5:12.5 (v/v), 2.5 mmol L^{-1} NaOH, 1 mmol L^{-1} sodium dodecyl sulfate). Chemical reaction of bases, hydroxide or met oxide ions, with trinitroaromatic compounds forms red derivatives. The color was detected by use of a green LED on the microchip. Two surfactants bearing opposite charge, cetyltrimethylammonium bromide (CTAB) and SDS were compared with regard to their effect on separation times, detection limits, and resolving power. All microchip separations were achieved in $<20\text{ s}$. When microchip separation was coupled with ex-situ solid-phase extraction the detection limits for these three explosives in seawater were: TNB, $0.25\text{ }\mu\text{g L}^{-1}$; TNT, $0.34\text{ }\mu\text{g L}^{-1}$ and tetryl, $0.19\text{ }\mu\text{g L}^{-1}$.

Soh et al. [42] developed a surface plasmon resonance (SPR) sensor for 2,4-dichlorophenol, a known precursor of dioxins. The SPR [43] sensor was applied in biological measurements of a variety of important compounds. In this report it was used to monitor 2,4-dichlorophenol by use of an indirect competitive immunoassay. The SPR sensor was fabricated by immobilizing a thin gold layer on the surface of an SPR sensor chip with an anti-(2,4-dichlorophenol) antibody using a gold binding polypeptide (GBP) and protein G. The SPR response based on the antigen-antibody reaction in a flow system was measured by injecting a 2,4-dichlorophenol sample solution into the flow system in which the SPR sensor was located. In a direct immunoassay system using the modified sensor chip, no significant SPR angle shift less than 0.001° was observed for 25 ppm 2,4-dichlorophenol solutions, because of the insufficient mass near the surface of the gold. To

improve the sensitivity of the SPR sensor 2,4-dichlorophenol was conjugated with bovine serum albumin (BSA) to make a large molecule for an indirect competitive immunoassay method. In the assay 350-ppm 2,4-dichlorophenol-BSA conjugate solutions containing different concentrations of 2,4-dichlorophenol (10–250 ppb) were injected into the SPR sensor system. The competitive immunoassay was found to be extremely sensitive. A detection limit of about 20 ppb was estimated. Verification that use of GBP to immobilize the antibody on the sensor chip enhanced the sensitivity to 2,4-dichlorophenol was obtained by comparing the procedure with another modification, in which BSA was used instead of GBP for immobilizing the antibody on the sensor chip. The affinity constant of 2,4-dichlorophenol and its conjugate to the antibody were estimated from the SPR response. Although this report describes application of SPR to an immunosensor, it will be readily applicable as a universal detection method for microchip separation. Sample needs not be fluorescent or UV-visible light absorptive, but should have a refractive index different from that of the carrier solution. Moreover, because SPR probes a solution in a very limited region in the vicinity of the gold surface (less than 1 μm ; twice the wavelength of the probe beam), sensitivity in SPR measurement is constant, irrespective of the size, especially the depth, of the microchip. The method will therefore be a good choice for the detection of large molecules such as proteins, polysaccharides, or their derivatives.

The thermal lens method is a type of photo-thermal conversion spectroscopy, with high sensitivity. Kitamori and co-workers [44] developed a thermal lens microscope (TLM) that achieved the thermal lens method under the microscope for the first time. TLM has detection capability of approximately 10^{-8} (Abs) for a 10- μm light path length. This method is 1000 times more sensitive than absorption spectrophotometry. Under ideal conditions, determination of non-fluorescent molecules has also been achieved at the one molecule level [45, 46]. TLM is an ideal method of detection for microchip systems [47, 48, 49]. TLM, however, entailed use of a large-scale laser, many optical components, and a microscope on the isolation stand for optics, and so was a very large-scale detection system, had no portability, and needed special operating technique. A desktop-sized thermal lens microscope (DT-TLM) with high sensitivity and wide applicability has recently been developed [50]. The utility of the DT-TLM compared with that of the large TLM has been remarkably improved by simplifying the optics and optimizing the optical configuration. The quantitative limit of determination estimated from the standard deviation (2σ) and the value of detection limit from the signal-to-noise ratio $S/N=2$ were 1.1×10^{-8} and $8.27 \times 10^{-9} \text{ mol L}^{-1}$, respectively. These values are almost the same as for the large TLM. DT-TLM has excellent characteristics in terms of sensitivity, generality, convenience, and portability as a detector for microchip systems. In the thermal lens method if the analyte has photo-absorption labeling is unnecessary. Direct observation on the cell [51] or in the cell [52] is also possi-

ble. Application of thermal lens method to a variety of fields is expected.

Conclusions

The original methods of detection of samples separated on microchips were only LIF or special instrumentation. After rapid developments in microfabrication technology and their analytical applications, however, researchers can now choose any detection method suitable for their purpose. Progress in fabrication technology and detection methods connected with separation techniques are inseparable from the widespread application of micrototal analysis. Further progress is expected in the field to make the analytical system more versatile and sensitive and to increase throughput.

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