Laser-Induced Fluorometry for Capillary Electrophoresis

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Abstract: Laser-induced fluorometry (LIF) has achieved the detection of single molecules, which ranks it among the most sensitive of detection techniques, whereas capillary electrophoresis (CE) is known as a powerful separation method with resolution that is beyond the reach of many other types of chromatography. Therefore, a coupling of LIF with CE has established an unrivaled analytical technique in terms of sensitivity and resolution. CE-LIF has demonstrated excellent performance in bioanalytical chemistry for the high-resolution separation and highly sensitive detection of DNAs, proteins, and small bioactive molecules. This review describes the CE-LIF methods developed by the author's group that include indirect and direct detection using diode lasers, post-column derivatization, and Hadamard transformation, as well as applications to the binding assays of a specific DNA immunoassays of proteins, and to the determinations of anticancer drugs.
1. Introduction

Laser-induced fluorometry (LIF) is recognized as an extremely sensitive detection method with a level of sensitivity that can detect a specific molecule at single-molecule levels. LIF features lasers that are monochromatic, directional, and coherent. These properties allow the laser beam to focus with a radius that ranges from a few micrometers to sub-micrometers. Therefore, LIF can detect volumes that range from nano-liters to pico-liters, as demonstrated in single-molecule detection.¹

Coupling LIF detection with high-performance liquid chromatography² was proposed in 1980 when capillary electrophoresis (CE) was still in its evolutionary phase. A primary CE method was reported in 1978 by Mikkers et al.³ followed by pioneering work in 1981 by the Jorgenson group.⁴ Obviously, LIF is compatible with CE wherein a small volume of a sample is injected into a narrow capillary with an inner diameter of less than 100 µm. Since the laser beam can be focused onto a spot that is smaller than the inner diameter of the capillary, fluorescence is efficiently collected by excluding scattered light via the use of spectral and special filters that result in an extremely low level of background signals. In 1985, when CE technology was still in its early stages, CE-LIF was first used in the detection of dansylated amino acid enantiomers using a helium-cadmium laser.⁵

Many biomolecules are inherently non-fluorescent in the visible region, and, therefore, they usually are labeled with a fluorescent or fluorogenic tag. For example, double-stranded DNA is usually labeled with an intercalator that generates enhanced fluorescence via binding with DNA molecules whereas proteins are frequently conjugated with a fluorescent labeling reagent, a fluorogenic labeling reagent, or a fluorescent protein. We can select one of many fluorescent or fluorogenic labeling reagents that are excited by lasers with different emission wavelengths of ultraviolet to near-infrared regions.
Conversely, the detection of target molecules labeled with a fluorescent labeling reagent requires a separation of the conjugate from the free fluorescent labeling reagent. The high resolution of CE satisfies the requirements for the selective detection of target molecules, so CE-LIF is an optimal combination that results in a high-performance analytical technique. In fact, the limits of detection for CE-LIF range from nM to pM levels, depending on the type of the fluorescent moiety and the detection system.

In this paper, our group's work with CE-LIF is reviewed from the viewpoints of fundamental study and application. We have developed novel CE and micellar electrokinetic chromatography (MEKC) methods coupled with LIF detection using diode lasers, post-column reactions, and Hadamard transformation. We also demonstrated some practical applications of CE-LIF and MEKC-LIF in a binding assay of DNA, immunoassays of proteins, and determinations of anti-cancer drugs.

2. Overview of CE-LIF

The use of CE-LIF and MEKC-LIF grew rapidly in the 1990s to 2000s. The number of publications on CE-LIF or MEKC-LIF listed in the Web of Science is shown in Fig. 1. The number of the publications indicates the articles containing the keywords “laser-induced fluorescence” and “capillary electrophoresis” or “micellar electrokinetic” in the title. In the years from 1988 to 2017, these topics were featured in 879 publications. In addition to these articles, the keywords “DNA sequencing” and “capillary electrophoresis” produced 150 more targeted publications, which totals more than 1,000 related publications in 30 years. As shown in Fig. 1, the increases in the number of publications reached a maximum in 2002, and then gradually decreased year by year. Decreases in the number of publications can be attributed to recognition of CE-LIF as a popular and conventional method rather than to a decrease in the uses, which resulted in
overlooking the need to list the keywords in the titles of articles.

A standard alignment for LIF detection in CE is shown in Fig. 2. In this alignment, a laser beam is directly focused on the detection window of a separation capillary where a polymer coating is usually removed by flame. Fluorescent molecules migrating in the capillary are excited by the laser beam and generate fluorescence, which is efficiently collected by a lens or a microscope objective. The collected fluorescence passes through optical and spatial filters to exclude scattered light caused by Rayleigh and Raman scattering, and the fluorescence is then converted to electric signals by a sensing device such as a photomultiplier tube, a photodiode, or a charge coupled device.

2. Diode LIF detection in CE

In LIF, any laser can be used for an excitation light source by selecting the appropriate labeling reagent and optical filters to remove scattered light. Diode lasers are popular light sources for use in reading memory devices such as audio CDs and DVDs, and diode lasers are compact, inexpensive, and require minimal maintenance compared with gas lasers and large solid lasers such as Nd:YAG lasers and Ti:sapphire lasers. The utility of diode lasers in fluorometry was first proposed in 1984 while gas lasers such as argon ion lasers and helium-cadmium lasers were frequently employed in molecular spectroscopy. Prior to the 21st century, the emission wavelengths of diode lasers were limited to a region longer than 600 nm, and such long wavelengths were considered both an advantage and a disadvantage. A long wavelength was recognized as suitable for bioanalysis because of the low background due to fewer native fluorescent molecules in the deep-red region, although conventional labeling reagents were not yet available in such long wavelengths. Therefore, some researchers focused on the development of labeling reagents that can be excited by diode lasers that emit in deep-red to near-infrared regions.
In the first demonstration of CE-LIF, helium-cadmium lasers emitting at 325 nm were a useful light source in LIF since the emission wavelength was suited to the excitation of dansylated molecules that are easily produced by the labeling reaction between dansyl chloride and primary amino groups. We also employed a helium-cadmium laser to determine dansylated aliphatic amines and polyaromatic hydrocarbons by MEKC-LIF. However, helium-cadmium lasers have disadvantages such as high cost and a short lifetime. These disadvantages can be mitigated by using a second harmonic wave from a diode laser, although the wavelength is not as short as that of a helium-cadmium laser.

A method to solve the problem of a long wavelength is the use of indirect detection wherein a fluorophore is added to a background electrolyte (BGE) of CE. In capillary zone electrophoresis (CZE), the indirect detection mechanism can be explained as the replacement of a charged fluorophore with charged analytes in the analyte zones based on the principle of electroneutrality. Conversely, in the indirect detection of electrically neutral analytes separated by MEKC, the mechanism is obviously different from that of CZE. The first demonstration of indirect detection in MEKC was reported by Amankwa and Kuhr, but a response mechanism was not proposed in their research. We also developed the indirect LIF detection of aromatic hydrocarbons separated by MEKC using a cationic surfactant and a fluorescent dye, oxazine 750, which can be excited by a diode laser emitting at 670 nm. The detection mechanism was explained by the replacement of the dye with analytes on the micelles that enhance the fluorescence intensity of the dye. Therefore, when the analytes exclude the dye molecules from the micelles, negative peaks appear on the baseline of high-background fluorescence. The indirect detection method was applicable to the detection of aromatic hydrocarbons with polar functional groups such as amino, nitro, and hydroxyl groups.

However, the sensitivity of indirect detection is actually much poorer than direct
detection due to the fluctuation of the high-background fluorescence. Obviously, direct
detection is more practical for the trace analysis of biological molecules than indirect
detection. In the direct detection of biomolecules, we needed fluorescent labeling
reagents that could absorb deep red light. There were a few labeling reagents with
fundamental molecular structures of cyanine dyes and thionine dyes that could be excited
by deep red lights. Cyanine dyes were successfully employed in the determination of
amino acids\textsuperscript{17} and their enantiomers.\textsuperscript{18} As light sources, a diode laser emitting at 635 nm
and a diode laser-pumped Nd:YAG laser emitting at 532 nm (second harmonic wave) were
employed in the detection of amino acids labeled with cyanine dyes.\textsuperscript{17}

Furthermore, the labeling reagent of cyanine dye was applied to the labeling of
proteins. However, in protein analysis using the labeling reagents, multiple labeling is a
serious problem because proteins contain more than two reactive amino groups in the
molecule, which results in different conjugates depending on the number of amino groups.
Thus, we optimized the conditions for the labeling reaction between a protein and a
cyanine labeling reagent and evaluated the effect of multiple labeling from the theoretical
plate number of the labeled protein. The height equivalent of one theoretical plate
(HETP) increased when the protein was multiply labeled, which indicates that HETP
represented the heterogeneity of the labeled protein.\textsuperscript{19} Furthermore, we used MEKC-LIF
to investigate the labeling reaction of the cyanine labeling reagent with bovine serum
albumin (BSA)\textsuperscript{20} and also evaluated the number of the fluorescent molecules that binded
with the BSA.\textsuperscript{21}

As the light source for LIF, diode lasers are superior to gas alternatives such as argon
ion or helium-cadmium lasers and offer a miniaturized, less-expensive system that has a
longer lifetime and requires less maintenance. Conversely, the labeling reagents suitable
for diode lasers have a relatively large molecular weight that leads to low reactivity due to
steric hindrance and a low frequency of collisions with analyte molecules. More recently, however, blue diode lasers emitting at 405 nm have become commercially available, so diode lasers now cover a wider region of wavelengths that range from blue to red.

When using blue and ultraviolet lasers, post-column derivatization coupling with a fluorogenic-labeling reagent can simplify the sample preparation. Fluorogenic reagents change the fluorescent properties by conjugating with analytes, which results in selective detection of the labeled analytes without separation of the free label, as opposed to pre-column derivatization that requires the reaction of analytes with a fluorescent labeling reagent prior to CE analysis and a separation of the free label from the labeled analyte for CE separation. In other words, the labeled analytes are spectrally separated from the labeling reagent. Therefore, post-column derivatization permits direct injection of a sample solution without pre-labeling of the analytes, which means CE separation using post-column derivatization reflects the native electrophoretic mobilities of the analytes whereas pre-column derivatization changes the electrophoretic mobilities of the analytes due to conjugation of the fluorescent label that adds an electric charge and mass to the analytes. Conversely, post-column reactors may lead to band broadening in the mixing step with the derivatizing solution, resulting in degradation of the resolution. Therefore, it is important to minimize the band broadening that originates from the dead volume of the reactor in a post-column reaction.

There are some reports on the use of post-column reactors in CE, and we have demonstrated different types of post-column reactors. The post-column reactors developed by our group are shown in Fig. 3. One consists of a laser-drilled capillary which has a small hole with a diameter similar to the inner diameter of the capillary, and another uses the sheath flow of a labeling reagent. Initially, the post-column reactor consisting of a laser-drilled capillary was employed in fluorescence detection using an
ultraviolet light emitting diode (UV-LED) to detect amino acids derivatized by o-phthalaldehyde (OPA), which is a fluorogenic reagent used to form fluorescent derivatives via reaction with primary amines.\textsuperscript{26} UV-LED is applicable as a light source for fluorescence detection, although it possesses neither the ability to focus nor the intensity of lasers.

Therefore, we employed a diode laser emitting at 405 nm, which appeared around 2004, in our LIF system. The diode laser can excite proteins derivatized with naphthalene-2,3-dicarboxaldehyde (NDA), which is a fluorogenic reagent that is as common as OPA. Using the diode laser, we achieved the first post-column derivatization of proteins separated by capillary sieving electrophoresis (CSE), which permits the separation of proteins based on molecular mass.\textsuperscript{28} The separation medium of the CSE separation consists of buffer components and polyethylene oxide acting in the role of a sieving matrix. It should be noted that the buffer component must be absent of primary amine because of its reactivity to NDA while a primary amine, tris(hydroxymethyl)aminomethane, is conventionally employed as a buffer component in gel electrophoresis and CSE. Thus, we attempted to find a base that would not react with NDA and could function as a buffer component in a CSE separation of proteins. Consequently, we found that a tertiary amine, 2-(diethylamino)ethanol (DEAE), is a suitable base that can be used to adjust the pH of the sieving matrix. When using BGE containing 100 mM \(N\)-cyclohexyl-2-aminoethanesulfonic acid, DEAE, 0.1% sodium dodecyl sulfate (SDS), and 1.5% PEO (pH 8.6), six standard proteins treated by SDS could be separated according to their molecular mass. The BGE permitted the separations of standard proteins, proteins in a milk sample, and proteins in cancer cells by CSE, followed by post-column derivatization with NDA.\textsuperscript{29}

A post-column reactor using sheath flow was applied to two-color LIF to discriminate
a glycosylated protein from an unglycosylated protein.\textsuperscript{27,30} We employed two lasers emitting at 450 (diode laser) and 532 nm (diode laser-pumped Nd:YAG laser) that were focused on a capillary in the same position in order to simultaneously record two electropherograms. \textit{The system of two color excitations is shown in Fig. 3.} Two solenoid beam stoppers controlled by a shutter controller were located on the pathways of the lasers. Two laser beams were overlapped by a dichroic filter, followed by focusing on a point 750 µm away from the end of the separation capillary. One of two lasers irradiated the capillary when the beam stopper was alternately opened and closed at 2 Hz, and data acquisition of the fluorescence was synchronized with the opening and closing of the beam stoppers. Therefore, each electropherogram obtained by excitations of either 450 or 532 nm was recorded at 1 Hz of the sampling frequency.

The Nd:YAG laser excited concanavalin A labeled with tetramethylrhodamine (Rh-ConA) whereas the diode laser excited any proteins derivatized with NAD in a postcolumn reactor. When Rh-ConA was reacted with a glycosylated protein, a complex of Rh-ConA and the glycosylated protein generated a fluorescence signal at the same migration timeframe in both electropherograms obtained by 450-nm and 532-nm excitations. However, if an unglycosylated protein was reacted with Rh-ConA, no signal appeared at the migration timeframe of the unglycosylated protein in the electropherogram obtained by a 532-nm excitation due to a lack of complex formation. Therefore, a protein was assigned to a state of glycosylation if it showed peaks at the same migration timeframe in both electropherograms.

Via use of the proposed principle, we differentiated a glycosylated protein (thyroglobulin) from an unglycosylated protein (albumin) in the presence of Rh-ConA. The separation of thyroglobulin and albumin is shown in Fig. 4.\textsuperscript{27} In Fig. 4(a), the sample contained two proteins that could not be resolved in the BGE consisting of 50 mM
of borate buffer (pH 9.2), which resulted in a single peak. These proteins were detected via a 450-nm excitation by the post-column reaction with NDA and 2-mercaptoethanol while no peak was observed at a 532-nm level of excitation in the absence of Rh-ConA. Conversely, in the presence of Rh-ConA, which was pre-mixed with the protein mixture, the migration time of thyroglobulin was decreased by the complex formation with Rh-ConA, resulting in the separation of thyroglobulin and albumin in the electropherogram obtained by an excitation of 450 nm, as shown in Fig. 4(b). Furthermore, only the complexed thyroglobulin exhibited a peak in the electropherogram obtained by an excitation of 532-nm, and the peak indicated that thyroglobulin was binding with Rh-ConA.

3. Hadamard transform CE

To improve the limits of detection in CE, we developed a sensitive detection scheme for CE separations, which is referred to as the Hadamard transform (HT)-CE.\textsuperscript{31,32} In HT-CE, plugs of a sample solution and the BGE solution were sequentially injected into a capillary according to a pseudorandom binary sequence constructed of a Hadamard matrix. A schematic illustration of the principle for HT-CE is shown in Fig. 5. In practice, an electropherogram is encoded by a pseudorandom binary sequence according to an S matrix, which is constructed by removing the first row and the first column of a Hadamard matrix consisting of 1 and -1 and by replacing these with 0 and 1, respectively. Therefore, the (n-1)-order of an S matrix is constructed from the n-order of a Hadamard matrix. The pseudorandom binary sequence consists of 2n-1 elements that are constructed by repeating the first row twice and deleting the last element. The encoding and decoding of electropherograms is expressed by equations (1) and (2), respectively.

\[ [\eta] = [S] \times [E] \]  

(1)
\[ [E] = [S]^{-1} \times [\eta] \]  

In equations (1) and (2), \([E], [S], [\eta], \text{ and } [S]^{-1}\) make up a 1\(\times\)n matrix of an electropherogram, a n\(\times\)n S-matrix, a 1\(\times\)n matrix of an encoded electropherogram, and an inverse matrix of the n\(\times\)n S-matrix, respectively. It should be noted that \([E]\) in eq. 2 is actually different from that in eq. 1, because the \([E]\) in eq. 2 is the transformed data wherein noises are canceled and signals are integrated.

In HT-CE, a sample solution must be injected sequentially with an application of electric potential to accomplish an electrophoretic separation, so that the sample injection and the separation can be simultaneously executed in the separation capillary. We first developed a multiple injection method by using an optically gated injection. \(^{31}\) The experimental setup for an optically gated injection is shown in Fig. 6(a). The optically gated injection is based on the photo bleaching of analytes, which decompose efficiently under the irradiation of a strong laser light. \(^{33}\) Gating and probe laser beams are focused onto a separation capillary from different positions that maintain a constant distance from each other. The sample injection is controlled by irradiation from the gating beam, because fluorescent analytes are not injected while the gating beam irradiates the capillary whereas blocking of the gating beam produces an analyte plug the length of which is proportional to the amount of time the gating beam is blocked. Therefore, samples can be sequentially injected by controlling an open-and-close shutter placed in the optical path of the gating beam.

The most suitable fluorophore for optically gated sample injection would be fluorescein ion, which has a high quantum yield (>0.9) and decomposes easily. The electropherograms in Fig. 5 represent the actual electropherograms obtained by a single injection (a) and encoded by multiple injections with modulation.
of the shutter according to a binary sequence order of 255 (b), and inverse transformation of the encoded electropherogram (c). A sample solution containing fluorescein ion was injected into a capillary 256 times, which equals n+1. The encoded electropherogram was decoded by multiplying the inverse matrix of the S-matrix, which resulted in an electropherogram with an improved S/N ratio, because the signal was integrated when the noise was cancelled. In principle, the S/N ratio can theoretically be improved \((n+1)^{1/2}\)-fold via HT-CE by increasing the order of the S-matrix, whereas analysis time increases in proportion to the order.  

The analysis time required for obtaining the encoded electropherogram is given by the product of the injection time for a single plug and \(2n-1\). For example, when the injection time for the single plug is set to 0.5 s with an S-matrix order of 255, the analysis time would be 254.5 s (4.24 min). Using a high-order matrix and a high-power laser, 27 molecules of fluorescein ion were detected using HT-CE.  

The HT-CE has been applied to the separation of amino acids and amino acid enantiomers labeled with fluorescein isothiocyanate (FITC). When using cyclodextrin as a chiral selector, FITC-labeled glutamic acid enantiomers are clearly separated in the transformed data with enhanced S/N ratios. Furthermore, Hadamard transformation using optically gated sample injection was applicable to the MEKC separation of amino acids. Therefore, HT-CE with optically gated sample injection is useful for the sensitive detection of FITC-labeled analytes with no degradation of resolution in the separations.

We also developed electrokinetic injection in both microchip electrophoresis and CE since the optically gated sample injection is only applicable to fluorescent analytes that are easily bleached. Microchip electrophoresis can switch the flow channels so that sample injections are easily modulated by a pseudorandom binary sequence. We constructed a
simple electronic circuit to drive reed relays, which switch an electric field applied to the channels in a microchip. A standard gated-injection method was employed to inject a sample solution according to an S-matrix.

The reed relay system was also applied to a capillary format where we laser-drilled a hole in the separation capillary to function as an injection port. The experimental setup for electrokinetic injection using the laser-drilled capillary is shown in Fig. 6(b). The system is potentially applicable not only to laser-induced fluorescence detection but also to any detection methods including absorption spectrometry and electrochemistry. We employed the electrokinetic injection system for the separation of amino acids labeled with rhodamine B isothiocyanate (RBITC), as shown in Fig. 7. Clear peaks of RBITC, glutamic acid (Glu), and phenylalanine (Phe) are observed in the transformed data (Fig. 7(b)), although only Glu was detected in the electropherogram obtained by a single injection (Fig. 7(a)).

An advantage of HT-CE is its applicability to any samples in contrast to preconcentration techniques known well in CE including field-amplified sample stacking, isotachophoretic preconcentration, and sweeping, all of which are difficult to apply to samples that contain other ions in high concentrations. In addition, the number of sample injections has no influence on theoretical plate numbers and resolution between analytes, although increases in the number of sample injections prolongs the analysis time. However, irreducible migration time is more serious in HTCE than in conventional CE, since the signal intensity is obtained by the addition or subtraction of large signals in HT. Fluctuation of the migration time induces incorrect encoding of the electropherogram, resulting in an insufficient enhancement of the S/N ratio. Coupling using other mathematical techniques such as cross-correlation and Fourier transformation has been reported by other researchers and reviewed in a book. After our first publication
detailing the use of HT-CE, several research groups also reported how HT-CE improves detection sensitivity.\textsuperscript{47-51} Although specific instrumentation is needed to achieve multiple sample injections, HT-CE has shown promise for the determination of analytes at concentrations lower than the limits of detection in CE separation. More recently, however, a technique for achieving HT-CE using a commercially available CE instrument was reported by Trapp’s group,\textsuperscript{52} and now HT-CE can be conventionally employed in CE analysis by providing suitable software.

4. Applications of CE-LIF to bioanalysis

CE is suited for bioanalysis of samples in extremely small volumes. A noteworthy application of CE-LIF is DNA sequencing. Many research groups consider CE-LIF to be the most promising for high-throughput DNA sequencing.\textsuperscript{53-55} In fact, CE-LIF was used in the termination of the human genome project in 2000.\textsuperscript{56}

Important applications of CE include binding assays based on hybridization, immunological reactions, and enzymatic reactions. A diode laser emitting at 780 nm was employed to excite a cyanine-labeled DNA molecule with the complementary sequence of a target DNA. The cyanine-labeled DNA acted as the probe DNA for the target DNA by annealing to form the double-stranded DNA that had been separated from the single-stranded probe DNA by CE.\textsuperscript{55-57} The limits of detection for the probe DNA was estimated to be 8 nM.

Under the optimized labeling conditions, human serum albumin (HSA) was labeled using a cyanine dye with an absorption band of approximately 630 nm. The labeled HSA was employed as a probe in the competitive immunoassay of HSA using CE. CE-based immunoassay permitted the determination of HSA in the range of 0.02 to 1.0 mg mL\textsuperscript{-1} with limits of detection (LOD) at 0.02 mg mL\textsuperscript{-1}.\textsuperscript{58} Further improvements in the LOD were
achieved by using an on-column competitive reaction of the labeled HSA and native HSA with anti-HSA antibody, in which native HSA was injected as a long plug. The on-column reaction improved the LOD of the CE immunoassay to 0.005 mg mL\(^{-1}\), which corresponded to a 14-fold lower value than that in an off-column reaction.\(^{59}\)

In a series of studies on the uptake of anticancer drugs (anthracyclines) into cancerous cells, an immunoassay using CE-LIF was developed for the determination of multidrug-resistant protein 1 (MRP1), which acts as a pump to excrete drugs outside of cell bodies. Some anthracyclines with the same basic skeleton such as doxorubicin (DOX), epirubicin (EPI), idarubicin, and daunomycin are known as anticancer drugs and are directly excited by a 488 nm argon ion laser, which results in fluorescence. Therefore, we initially attempted to develop a method to simultaneously determine four anthracyclines via MEKC-LIF in order to clarify the uptake behavior of anthracyclines. Although separation of the anthracyclines was difficult because of their similar bone structure, they were successfully separated by MEKC using a BGE containing sodium taurodeoxycholate, (2-hydroxypropyl)-\(\gamma\)-CD and SDS.\(^{60,61}\)

The MEKC-LIF method is used to trace the amount of drugs incorporated into cancer cells RDES and A549. When these tumor cells were treated in a culture medium containing two anthracyclines, DOX and EPI, for fixed periods of time, intracellular concentrations could then be determined by directly injecting cell lysates. These results indicate that the uptake of EPI is slightly less than that of DOX for A549, but higher for RDES. Conversely, intracellular accumulation of anthracyclines was greater in RDES than in A549, but both cell lines excreted the anthracyclines after 12 h.\(^{59,60}\)

A decrease in the intracellular concentration of anthracyclines frequently results from the increased expression of MRP1. To clarify this assumption, we developed a CE immunoassay of MRP1 by using FITC-labeled anti-MRP1 as a probe. The FITC-labeled
anti-MRP1 was prepared by reacting anti-MRP with FITC, followed by purification with ultrafiltration. When an excess amount of the FITC-labeled anti-MRP1 was added to a cell lysate, a complex between MRP1 and FITC-labeled anti-MRP was formed after incubation at 37 °C for 1 h. The developed CE immunoassay showed that the expression of MRP1 had increased in both cell types, RDES and A549, after treatment with DOX for 12 h, and the amount of DOX in the cells was reduced. The increased expression of MRP1 was correlated with the decrease of DOX in the cells. These studies are reviewed in an article of related work.

4. Conclusions

Recent advances in laser and spectroscopic technologies have established a revolution in spectroscopy. For example, blue diode lasers were unavailable only twenty years ago, and now these are a practical part of life. Many sensitive photodetectors such as photomultiplier tubes, photodiodes, and charge-coupled devices were developed in spectroscopic imaging. These technologies have been used for detection at the single molecule level by coupling with the appropriate chemistry. In the 1980s, CE had a strong impact on chromatographic and electrophoretic research because of its extremely high resolution. Since the probed volume in CE is limited to several nano-litters, LIF is compatible with CE and results in ultra-sensitive detection. The CE-LIF method attracted many researchers during the late 1990s to early 2000s, particularly in fields that depended on biochemical analyses. Recently, the technique has been established in many fields beyond bioanalytical chemistry such as environmental and materials sciences. However, CE-LIF will remain a matter of research because new technologies are developing in optics and chemistry. For example, diode lasers may expand the emission wavelength to deep ultraviolet regions, and a new functional molecule may represent an
excellent fluorescence property that is yet to be achieved. Such technologies will make CE-LIF more attractive and make it possible to realize chemical analyses powered by ever-greater levels of high-resolution separation and highly sensitive detection. Obviously, CE-LIF is expected to be an analytical technique that will be used to realize the highest possible levels of separation and sensitivity.

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References


Figure Legends

Fig. 1 Publications on CE-LIF and MEKC-LIF.

Fig. 2 Schematic illustration of an LIF detector for CE.

Fig. 2 Schematic illustration of post-column reactors for CE.

Fig. 3 Schematic illustration of a two-color CE-LIF system. Reproduced from Ref. 27 with permission. Copyright (2013) Wiley

Fig. 4 Electropherograms of a mixture containing thyroglobulin and albumin.
(a) 4.9 µM thyroglobulin and 49 µM albumin, (b) 4.9 µM thyroglobulin and 49 µM albumin with 4.9 µM Rh-Con A; BGE, borate buffer (pH 9.2); derivatization solution, 1 mM NDA, 8 mM 2-mercaptoethanol, and 30% methanol in 70 mM borate buffer (pH 9.2). Reproduced from Ref. 27 with permission. Copyright (2013) Wiley.

Fig. 5 Principle of HT-CE.
(a) Electropherogram obtained by single injection, (b) Electropherogram obtained by Hadamard transformation, and (c) transformed data of (b). Sample, 10 nM sodium fluorescein in 10 mM KHCO₃ + NaOH (pH 9.3).

Fig. 6 Experimental setup for HT-CE.
(a) With optically gated injection and (b) with electrokinetic injection. Reproduced with permission from Ref. 31 and 40. Copyright (1999 and 2004) American Chemical Society.

Fig. 7 Electropherogram and transformed data of a mixture containing RBITC-labeled amino acids.
(a) Electropherogram obtained by single injection and (b) transformed data obtained by HT-CE. BGE, borate–Tris (pH 9.0); effective length, 32 cm; total length, 60 cm; electric field, 150Vcm⁻¹; labeling reagent, rhodamine B isothiocyanate (RBITC): concentrations of phenylalanine (Phe) and glutamic acid (Glu), 1.9 nM. Reproduced from Ref. 41 with permission. Copyright (2006) Elsevier.
Figure 1
Figure 2

(a) Separation Capillary

Fluorogenic Reagent

Hole (Fabricated by Laser Ablation)

Microcentrifuge Tube

Detection Window

Laser

(b) Separation Capillary

Tee Connector

Fluorogenic Reagent

Sheath flow contains the fluorogenic reagent

Detection Window

Laser

Large-bore Capillary
Figure 3
Figure 4

(a)

(b)
(a) Single Injection

\[ \begin{bmatrix} S \end{bmatrix} \times \begin{bmatrix} E \end{bmatrix} \]

(b) Multiple Injections

\[ \begin{bmatrix} S \end{bmatrix}^{-1} \times \begin{bmatrix} \eta \end{bmatrix} \]

(c) Inverse Hadamard Transformation

\[ \begin{bmatrix} S \end{bmatrix}: \text{Pseudorandom Square Matrix} \]

Figure 5
Figure 6

High Voltage Power Supply
Personal Computer
Detector
Reed Relay
9.0 kV
7.5 kV
Pt(+) Pt(-)...

Argon Ion Laser
Capillary
Gating Beam
Probe Beam
Pinhole Notch Filter
Shutter
Beam Splitter

(a)

(b)
Figure 7

Fluorescence Intensity

Time / s

Glu

Phe

RBITC

(a)

Fluorescence Intensity

Time / s

Glu

(b)