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# Capillary electrophoresis coupled with laser-induced fluorescence polarization as a hybrid approach to ultrasensitive immunoassays

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**Abstract**

Immunoassays using capillary electrophoresis with laser-induced fluorescence detection (CE–LIF) is a powerful approach to the determination of trace amounts of analytes in a complex biological matrix. However, its applicability is limited by the requirement that the free and bound tracer (fluorescently labeled compound) be resolved for their identification and quantitation. Here we show that replacing LIF with laser-induced fluorescence polarization (LIFP) permits ultrasensitive immunoassays to be performed with or without the separation of the free and bound tracer. A binding system involving cyclosporin A (CyA) and monoclonal antibody to CyA was chosen to demonstrate both homogeneous and heterogeneous immunoassay approaches. In the homogeneous scheme where the free and bound tracer were not separated, the fluorescence polarization of the mixture was a quantitative measure of the antibody-bound tracer. The concentration and mass detection limits for CyA using the homogeneous competitive assay were found to be 1 n*M* and 1 amol ($10^{-18}$ mol), respectively. The heterogeneous assay involved a nearly baseline separation of the free and bound tracer using CE with a phosphate running buffer of pH 7.0. The complex of the tracer with the antibody had a fluorescence polarization of approximately 0.24 whereas the free tracer had negligible polarization. The fluorescence polarization was independent of analyte concentration, and the fluorescence intensity of either the free or bound tracer was used for quantitation. Results from both assays suggest that the CE–LIFP approaches may have a wider application than the immunoassays based on either CE–LIF or fluorescence polarization alone. 

*Keywords:* Immunoassays; Laser-induced fluorescence polarization detection; Detection electrophoresis; Fluorescence polarization; Cyclosporins; Monoclonal antibodies

## 1. Introduction

Biotechnology and biomedical research have benefited from the development of highly selective and sensitive analytical techniques. Immunoassay is such a technique that has become prominent in all key areas of biologically related research. Its applications range from clinical analysis and environmental studies to gene expression detection [1,2]. The popularity arises largely from the specificity of the immunorecognition of an antibody for its target molecule and the sensitivity of various detection schemes using radioactivity, fluorescence, and enzymatic amplification introduced by appropriate labeling techniques. The current trend is toward the use of non-radioactive labels and the miniaturization of assays to provide analyses with smaller sample volumes, reduced reagent consumption and faster analysis while retaining the sensitivity in nanomolar range. One approach to this goal is to combine

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immunoassays with highly efficient separation and detection techniques such as capillary electrophoresis with laser-induced fluorescence (CE–LIF).

CE–LIF is an attractive technique for immunoassays because the narrow-bore capillaries provide high-speed, high resolution separations and allow ultrasensitive detection to be achieved in a minimal detection volume. The use of CE–LIF for rapid immunoassay was first demonstrated in 1993 by Schultz and Kennedy [3]. They separated the free and antibody-bound species of the fluorescein isothiocyanate (FITC) labeled insulin within 3 min, and obtained detection limits of 50, 280, and 420 zmol ($10^{-21}$ mol) for FITC labeled insulin, monoclonal anti-insulin, and unlabeled insulin, respectively. Since then, the CE–LIF technique has been explored extensively and applied to the determination of a variety of compounds including therapeutic drugs, peptides, and antibodies [4–6].

Despite the demonstrated potential, the CE–LIF based immunoassays require the separation of the free and bound species of the labeled compound used as a tracer. It is conceivable that, in some cases, the free and bound tracer may have similar electrophoretic mobilities and thus cannot be separated, making the technique useless in their identification and quantitation. To alleviate this problem, an elaborate approach was proposed involving the attachment of a charge modulator to either the tracer or the antibody and consequently the alteration of its electrophoretic mobility [7,8]. This approach has shown to be effective in improving the separation, however, the procedure involving chemical derivatization is tedious, time-consuming and difficult to control. Here, we show that by replacing LIF with laser-induced fluorescence polarization (LIFP), the technique can be used to perform ultrasensitive immunoassays with or without the separation of the free and bound tracer. Compared to conventional fluorescence polarization immunoassay (FPIA) [9,10], our approach has led to a substantial reduction in detection volume and a significant improvement in mass detection sensitivity. In addition, the CE–LIFP technique can be used to perform heterogeneous assays when the separation of the free and bound tracer are possible. In this case, the role of the CE–LIFP is similar to that of the CE–LIF but the fluorescence polarization provides additional information for the identification of affinity complexes.

To evaluate the CE–LIFP approach, we conducted two types of competitive immunoassays: homogeneous and heterogeneous. In the homogeneous assay, the fluorescent sample containing free and antibody-bound tracer was transported and delivered into the detection cell by electrokinetic pumping. A decrease in fluorescence polarization with addition of unlabeled analyte was measured, which serves as a basis for identification and quantitation. The heterogeneous assay was based on separation of the free and bound tracer. The fluorescence polarization was used to identify the bound tracer whereas the change in fluorescence intensity for either free or bound tracer due to competitive binding of antibody with unlabeled analyte was used for quantitation purposes. The data presented demonstrate the feasibility of performing both types of immunoassays by the CE–LIFP technique. Major advantages over the earlier techniques include lower mass detection limits, enhanced identification capacity, and the potential for further miniaturization.

## 2. Theory of fluorescence polarization

A simple model for fluorescence polarization is to consider two linear dipole oscillators held together at a fixed angle with one of the oscillators corresponding to absorption and the other to emission [11]. The degree of polarization of fluorescence at right angles to the incident beam is determined by the value of the angle between the absorption and emission oscillators. The emission has maximum polarization when the two oscillators are parallel to each other whereas the emission has minimum polarization when the two oscillators are perpendicular to each other. If the excitation is due to vertically polarized light, the degree of polarization, $P$, is defined as

$$P = (I_v - I_h)/(I_v + I_h) \quad (1)$$

where $I_v$ and $I_h$ are the intensities of the vertically and horizontally polarized components of the emission.

The theory of fluorescence polarization was first proposed by Perrin [12] in 1926 for spherical molecules and was later extended by Weber [13] to the case of non-spherical molecules. Briefly, the

polarization of fluorescence is determined by the orientation of a molecule at excited state relative to the electric vector of the excitation. Maximum absorption takes place when the electric vector of the exciting light coincides with the direction of the transition moment in absorption. If this orientation remains unchanged during fluorescence emission, such as in crystals where the molecules are oriented parallel to each other, the emission has maximum polarization. In solution, however, depolarization occurs due to the loss of molecular orientation resulted from rotational diffusion. For the simple case of spherical molecules in solution, the molecule's rotational relaxation time, $\rho$, is related to parameters of both the fluorescent molecules and the solvents by

$$\rho = 3\eta V/RT \quad (2)$$

where $\eta$ and $T$ are the viscosity and temperature of the solution respectively, $V$ is the molar volume of the fluorescent solute, and $R$ is the gas constant. According to Perrin [12], the fluorescence emitted at the right angles to the direction of excitation by linearly polarized light will have the polarization, $P$, given by

$$(1/P - 1/3) = (1/P_0 - 1/3)(1 + RT\tau/\eta V) \quad (3)$$

where $\tau$ is the fluorescence lifetime and $P_0$ is the intrinsic polarization in the absence of rotational diffusion, which has a maximum value of 0.5.

The importance of fluorescence polarization as an analytical technique is its sensitivity to changes in molecular size as a result of chemical bonding or non-covalent binding with other molecules. According to Eq. (3), for example, a large molecule of bovine serum albumin labeled with a fluorescein isothiocyanate exhibits an increase in polarization with respect to the fluorescent dye itself. Similarly, if a small fluorescent molecule of low polarization, such as FITC labeled cyclosporin A, binds to a large molecule as in an antigen–antibody interaction, the polarization increases as a result of the increased molecular size and accompanying restriction of the molecular rotation. Thus, the measurement of fluorescence polarization offers a means to qualitative and quantitative analysis of molecular interactions without the need for physical separation of the interacting molecules.

## 3. Experimental

### 3.1. Instrumentation

The CE–LIFP based immunoassays were performed using an experimental setup similar to that described previously [14]. A high voltage power supply (Model CZE 100R, Spellman, Plainview, NY, USA) was used to drive the sample through a fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA), which was inserted in a sheath flow cuvette (NSG Precision Cells, Farmingdale, NY, USA). The cuvette was illuminated with a vertically polarized laser beam filtered through a laser line filter (488 nm, 10-nm band width, Newport, Fountain Valley, CA, USA) from a 65-mW argon ion laser (Model 2014-65ML, Uniphase, San Jose, CA, USA). The fluorescence was collected by a 60× microscope objective lens (0.7 NA, Universe Kogaku, Oyster Bay, NY, USA). The fluorescence intensities corresponding to vertically and horizontally polarized emissions were measured, respectively, by two photomultiplier tubes (PMTs; R1477, Hamamatsu, Japan), after passing through a narrow bandpass filter (515 nm, 10-nm band width, Newport) and a pinhole and separated by a polarizing cube beamsplitter (03 PBS 023, Melles Griot, Irvine, CA, USA). The operation of the power supply and the acquisition of data were controlled by a Power Macintosh computer with an application software written in LabView (National Instruments, Austin, TX, USA).

### 3.2. Materials and reagents

Disodium fluorescein of purified grade was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Fluorescein labeled cyclosporin A (CyA; tracer) and monoclonal anti-CyA solutions were obtained from Abbott Labs (Chicago, IL, USA) as part of a TDx test kit provided for FPIA analysis of CyA. CyA and FPIA dilution buffer were obtained from Sigma (St. Louis, MO, USA).

### 3.3. Procedures

#### 3.3.1. Tracer/antibody complexation

Various volumes (0, 2, 4, 6, 8, 10, 15 and 20 μl) of solution S (antibody) from a test kit for CyA were mixed with 10 μl aliquots of solution T (tracer) in

0.5 ml microcentrifuge tubes. FPIA dilution buffer was added to each tube to bring a total volume to 300 μl. The tubes were vortexed for 30 s and allowed to incubate at room temperature for 15 min. The samples were analyzed by CE–LIFP.

*3.3.2. Competitive assay*

CyA standard solutions (0, 0.1, 0.25, 0.5, 1, 2 and 4 μg/ml) were prepared by serial dilution of a methanol solution of CyA (1000 μg/ml) with FPIA dilution buffer. To microcentrifuge tubes were pipetted 6 μl aliquots of CyA standard solutions, 10 μl each of solution S and solution T from the test kit, and 274 μl of FPIA dilution buffer to give a total volume of 300 μl. After 15 min of incubation at room temperature, the samples were subjected to CE–LIFP analysis.

The assays developed were applied to measure CyA in whole-blood samples obtained from organ transplant patients. Each whole-blood specimen was treated with methanol (methanol–blood, 2:1), vortexed for 30 s, then centrifuged for 5 min. The clear supernatant was removed and used in homogeneous or heterogeneous immunoassays for CyA. To compensate for the matrix effects, calibrations were carried out by adding various amounts of CyA to a patient sample with a CyA concentration of 70 μg/l as determined by conventional FPIA method [15,16].

*3.3.3. CE–LIFP*

A capillary of 35 cm×20 μm I.D×148 μm O.D. was used throughout this work. The capillary was preconditioned periodically by rinsing with 0.1 *M* NaOH, ionized water and the running buffer successively to ensure that reproducible results could be obtained. Samples were electrokinetically injected into the capillary by applying an electric field of 143 V/cm for 5 s and transported or separated at an applied field strength of 714 V/cm.

The performance of the detector was optimized by aligning a tightly focused laser beam with a small-diameter sample stream and balancing signals from two PMTs. An aqueous solution of disodium fluorecein ($10^{-8}$ *M*) was passed through a capillary inserted in a sheath flow cuvette. The sheath fluid, identical to the run buffer, was introduced into the cuvette hydrodynamically by keeping the inlet reservoir of the sheath buffer 1 cm higher than the outlet reservoir [17–19]. The laser beam was focused onto a spot about 20 μm below the tip of the capillary. The angle and position of the cuvette relative to the detection optical path were adjusted so that equal signals with a maximum from both PMTs were observed.

The polarization bias introduced by the optical components of the detection system was compensated for by calculating polarization with use of the correction factor, $\alpha$:

$$P = (I_v - \alpha I_h)/(I_v + \alpha I_h) \quad (4)$$

where $\alpha$ is the intensity ratio of vertically polarized component to horizontal component for a reference compound such as fluorescein as used in this work. For a well-balanced system, the polarization bias is relatively small ($\alpha \sim 1$) as is the case for polarization data presented in this paper. Therefore, polarization was calculated using Eq. (1).

## 4. Results and discussion

### *4.1. Homogeneous assays*

Interest in coupling CE with LIFP for ultrasensitive immunoassays has followed from an appreciation not only of the separation power of CE but also its capability of nanoliter sample delivering. Electroosmotic pumping generated by applying a high voltage across a capillary provides a convenient way to transport and deliver interaction reagents and samples into the detection cell [20]. A distinct advantage of such pumping is that the fluid flow can be induced in miniaturized fluidic devices such as microchip and controlled without the need for valves or other moving parts [21–24]. Thus, the use of electroosmotic pumping as a sample introduction method should open up a possibility to perform fluorescence polarization assays in narrow bore capillaries or micromachined devices.

To test the feasibility of this concept, we carried out the measurement of fluorescence polarization for a series of solutions in which varying amounts of fluorescein labeled cyclosporin A (tracer) and antibody were mixed. The binding system involving cyclosporin A and its antibody was chosen because previous studies [14] showed that the relative migra-

tion of the free and bound tracer can be adjusted by changing the pH of the running buffer. With the running buffer of pH 10.5, the free and antibody-bound tracer comigrate so that the variation of fluorescence polarization with the tracer/antibody ratio can be easily observed.

Fig. 1 shows two typical electropherograms obtained simultaneously, with the upper and bottom traces corresponding, respectively, to vertically and horizontally polarized emissions. The free and bound tracer were coeluted at 2.6 min and the internal standard, fluorecein, was eluted at 4.7 min. The values of fluorescence polarization resulted from the formation of tracer/antibody complexes were calculated from areas of tracer/antibody peaks according to Eq. (1) and are shown in Fig. 2 as a function of the antibody/tracer ratio. With the addition of antibody, the polarization increases continuously, as expected because the small-size fluorescent tracer forms complex with the large-size antibody.

Fig. 3 shows a calibration curve that was constructed by titrating a mixture of the tracer and the antibody in 1:1 volume ratio with a series of unlabeled CyA solutions. The tracer and antibody solutions from the Abbott reagent kit were diluted 30× in FPIA dilution buffer (Sigma, St. Louis, MO, USA). The actual concentrations and the compositions of these solutions are not disclosed and therefore dilutions of the original solutions were indicated as relative concentrations throughout this work. The variation of polarization shown follows the behavior expected for competitive binding assays: an increase in the amount of CyA leads to a decrease in polarization as a result of increased dissociation of the tracer/antibody complexes. A linear dynamic range covers the concentrations from 0 to 10 ng/ml (8.3 n*M*). The concentration detection limit for CyA,

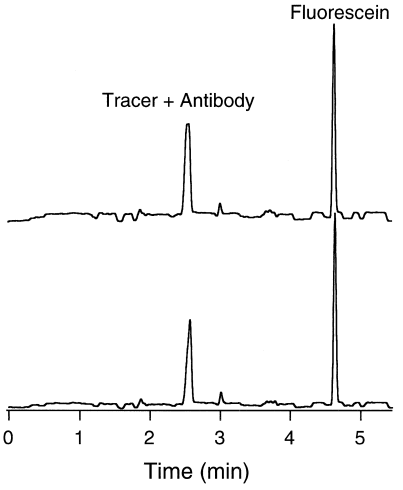


Fig. 1. Electropherograms of a mixture containing cyclosporin A tracer and its antibody in 1:1 volume ratio. Conditions: fused-silica capillary, 35 cm×20 μm I.D.; 0.01 *M* sodium tetraborate solution, pH 10.5; 20 kV; LIFP detection with excitation at 488 nm and emission at 515 nm. Upper and bottom traces correspond to vertically and horizontally polarized emissions, respectively.

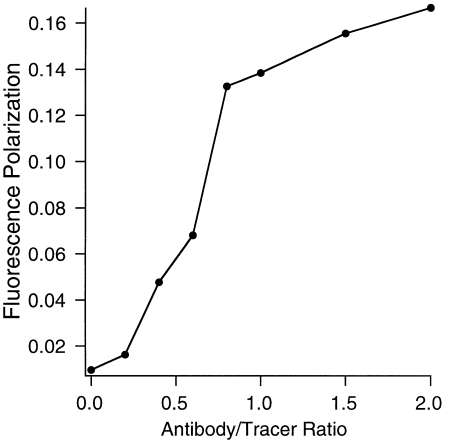


Fig. 2. Fluorescence polarization as a function of antibody/tracer ratio (●). Same conditions as shown in Fig. 1 were used.

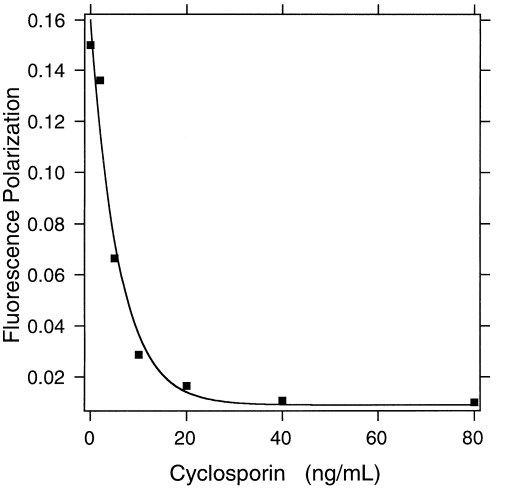


Fig. 3. Fluorescence polarization as a function of unlabeled cyclosporin A added (■). Same conditions as shown in Fig. 1 were used.

estimated as the concentration equivalent to the blank signal plus three-times the standard deviation from the replicate blank runs, was found to be on the order of 1 n*M*, which is about 20-fold lower than that possible with conventional fluorescence polarization assays [15,16]. The mass detection limit was 1 amol for an injection volume of approximately 1 nl. With a convenient working volume of 5–10 μl, the amount of sample needed is at least 10-fold smaller than that required by conventional polarization assays. These results demonstrate that, with the CE/FPIA approach, it is feasible to perform fluorescence polarization immunoassays in a miniaturized fluidic device, leading to a substantial reduction in mass detection limit as well as sample and reagent consumption.

It should be pointed out that the reported linear dynamic range and the detection limits are valid only under the conditions specified in this work. According to Eq. (1), an increase in detection sensitivity should be achievable, at the expense of the linear dynamic range, by decreasing the concentration of free tracer. This would require further optimization of the experimental conditions especially the fluorescent tracer concentration.

The homogeneous assay employing CE–LIFP was applied to the measurements of CyA levels in three patient samples. Based on a whole-blood matrix matched calibration, we obtained CyA concentrations in three samples: 95±13, 180±20, and 405±17 μg/l, respectively. For comparison, analyses of these blood samples by a clinical laboratory using a conventional FPIA [15] gave corresponding values of 89, 190, and 403 μg/l. The results from the two methods are in good agreement, confirming the applicability of our method to clinical analysis.

### *4.2. Heterogeneous assays*

With a role similar to that of the CE–LIF in the case where the free and bound tracer are separable, the CE–LIFP approach can also be used to quantify the individual fluorescent species based on their fluorescence intensities. An additional advantage of this approach is that large molecules such as antibody-bound tracer can be readily identified on the basis of their characteristic fluorescence polarization. Its utility in this context was demonstrated by performing an immunoassay for CyA under the conditions that the free and bound tracers were resolved.

As has been shown previously [14], CE separation of CyA tracer from its complexes with antibody can be achieved by lowering the running buffer pH. Fig. 4 illustrates such a separation with a phosphate running buffer of pH 7.0. The free and bound tracers are nearly baseline separated, with the complex being migrated first. It is noted that, while the intensities corresponding to the vertically and horizontally polarized emissions are approximately equal for the free tracer, the vertically polarized emission is significantly greater than the horizontally polarized component for the bound tracer. This feature was observed over a wide range of antibody/tracer ratios, from 0 to 2 (Fig. 5).

Based on the difference in intensity between two emission components, the fluorescence polarization was found to be 0.24±0.03 for the bound tracer and -0.07±0.02 for the free tracer. The relatively constant values of polarization at various antibody/tracer ratio is understandable because the polarization of a fluorescent molecule is primarily determined by its molecular size but not the amount present in a given system. The origin of the small polarization noted for the free tracer is unclear at

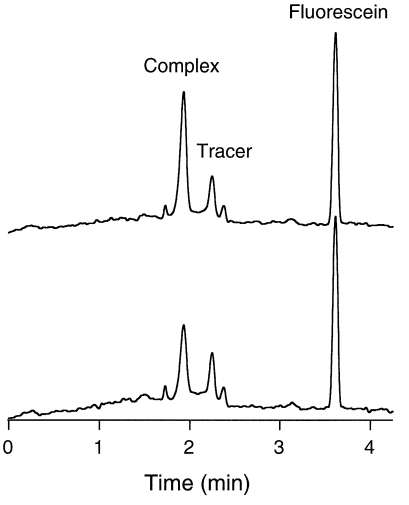


Fig. 4. Separation of a mixture containing cyclosporin A tracer and its antibody in 1:1 volume ratio. The running buffer was 0.02 *M* sodium phosphate solution at pH 7.0. Other conditions were the same as shown in Fig. 1.

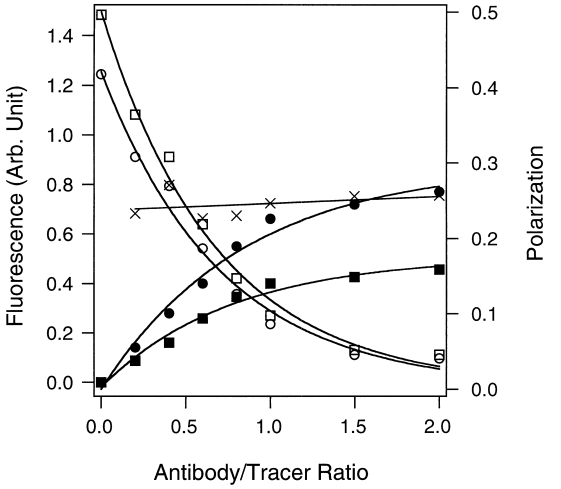


Fig. 5. Fluorescence intensity and polarization as a function of antibody/tracer ratio. CE–LIFP conditions were the same as for Fig. 4. (□) horizontally polarized emission of the free tracer; (○) vertically polarized emission of the free tracer; (■) horizontally polarized emission of the complex; (●) vertically polarized emission of the complex; and (×) polarization of the complex.

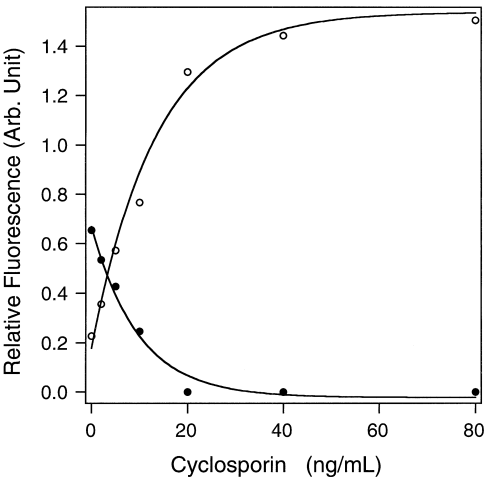


Fig. 6. Calibration curves obtained with increasing amounts of analyte cyclosporin A and fixed amounts of antibody and fluorescent tracer. CE–LIFP conditions were the same as for Fig. 4. (○) vertically polarized emission of the free tracer; (●) vertically polarized emission of the complex.

present and may be clarified in future work. From the practical point of view, however, the polarization of this magnitude is of no significance to analysis and therefore may be neglected thereafter.

While the polarization facilitates identification of the antibody/tracer complex in heterogeneous assays, it cannot be used for quantitative purposes. Instead, the fluorescence intensity is measured and a calibration curve is constructed by titrating a mixture of the tracer and antibody with the analyte CyA. Four calibration curves can be obtained, corresponding to the vertically and horizontally polarized fluorescence emitted by the free and bound tracer, respectively. Although each of them can be used for quantitation of CyA, preference is given to the one that shows higher sensitivity and lower interference. Fig. 6 shows the vertical component of fluorescence intensity from the free and bound tracer as a function of CyA concentration. Similar calibration curves were obtained for the determination of CyA in patient blood samples.

An application of the heterogeneous CE–LIFP assay was demonstrated for the determination of CyA in three whole blood samples from organ transplant patients. A whole blood sample was used as the matrix to construct the calibrations. Using the external calibration, the concentrations of CyA in the samples were found to be $95\pm14$, $185\pm18$ and $396\pm23$ μg/l, respectively. These data are in good agreement with the corresponding values of 89, 189, and 403 μg/l as determined using a conventional FPIA method [15].

## 5. Conclusions

A limiting factor to lower detection limits of fluorescence immunoassays is often due to scattered light and background fluorescence from the solvent and sample matrix. A simple way to reduce this background is to minimize the detection volume and to separate the impurities from the analytes [25]. This can be achieved readily using capillary electrophoresis with laser-induced fluorescence. A drawback of this approach is the requirement for separation of the free and bound tracer, which may not be always possible in practical applications. In contrast, fluorescence polarization allows a homogeneous assay to be performed without having to separate the free from the bound tracer. However, the conventional fluorescence polarization (FP) method has the disadvantages of large detection volume and low

mass detection sensitivity. We showed in this work a hybrid approach involving a combination of CE with laser-induced fluorescence polarization which allowed both heterogeneous and homogeneous assays to be performed on the same experimental setup with an improved mass detection sensitivity. Combining the advantages of the separation power and nanoliter sample delivering capability of capillary electrophoresis with the exquisite sensitivity and sample identification capacity of laser-induced fluorescence polarization, this approach has potential for a broader application than the CE–LIF based heterogeneous and FP based homogeneous immunoassays alone. Additional fluorescence polarization information can assist in identification of target molecules from a complex matrix. Thus, CE–LIFP offers a distinct advantage over other schemes in monitoring enzymatic digestion and fluorescent labeling reaction, and in screening for specific antibodies.

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## References

[1] R.M. Nakamuro, Y. Kasahara, G.A. Rechnitz (Eds.), Immunochemical Assays and Biosensor Technology for the 1990s, American Society for Microbiology, Washington, DC, 1992.
[2] M. De Frutos, S.K. Paliwal, F.E. Regnier, Methods Enzymol. 270 (1996) 82–101.
[3] N.M. Schultz, R.T. Kennedy, Anal. Chem. 65 (1993) 3161–3165.
[4] J.J. Bao, J. Chromatogr. B 699 (1997) 463–480, and references cited therein.
[5] N.H.H. Heegaard, S. Nilsson, N.A. Guzman, J. Chromatogr. B 715 (1998) 29–54, and references cited therein.
[6] D. Schmalzing, W. Nashabeh, Electrophoresis 18 (1997) 2184–2193, and references cited therein.
[7] F.T.A. Chen, J. Chromatogr. A 680 (1994) 419–424.
[8] F.T.A. Chen, J.C. Sternberg, Electrophoresis 15 (1994) 13–21.
[9] W.J. Checovich, R.E. Bolger, T. Burke, Nature 375 (1995) 254–256.
[10] D.M. Jameson, W.H. Sawyer, Methods Enzymol. 246 (1995) 283–300.
[11] G.G. Hammes, C. Frieden, L.W. Nichol (Eds.), Protein–Protein Interactions, John Wiley & Sons, New York, 1981, pp. 262–266.
[12] F. Perrin, J. Phys. Radium 7 (1926) 390–401.
[13] G. Weber, Adv. Protein Chem. 8 (1953) 415–459.
[14] L. Ye, X.C. Le, J.Z. Xin, M. Ma, R. Yatscoff, J. Chromatogr. B 714 (1998) 59–67.
[15] R.W. Yatscoff, K.R. Copeland, C.J. Faraci, Clin. Chem. 36 (1990) 1969–1973.
[16] P.P. Wang, E. Simpson, V. Meucci, M. Morrison, S. Lunetta, M. Zajac, R. Boeckx, Clin. Biochem. 24 (1994) 55–58.
[17] Y.F. Cheng, N.J. Dovichi, Science 242 (1988) 562–564.
[18] S. Wu, N. J Dovichi, J. Chromatogr. 480 (1989) 141–155.
[19] X.C. Le, C. Scaman, Y. Zhang, J. Zhang, N.J. Dovichi, O. Hindsgau, M.M. Palcic, J. Chromatogr. A 716 (1995) 215–220.
[20] Q.H. Wan, Anal. Chem. 69 (1997) 361–363.
[21] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Science 261 (1993) 895–897.
[22] A. Manz, D.J. Harrison, E.M.J. Verpoorte, H.M. Widmer, Adv. Chromatogr. 33 (1993) 1–66.
[23] N. Chiem, D.J. Harrison, Anal. Chem. 69 (1997) 373–378.
[24] S.C. Jacobson, J.M. Ramsey, Anal. Chem. 68 (1996) 720–723.
[25] P.M. Goodwin, W.P. Ambrose, R.A. Keller, Acc. Chem. Res. 29 (1996) 607–613.