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Short communication

Development of a compact chemiluminescence system coupled with capillary electrophoresis for carbohydrate analysis

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ABSTRACT

As a kinetic process, chemiluminescence (CL) met great challenges while it was used in the detection methods coupled with capillary electrophoresis (CE). In this investigation, a newly recorded ultra-fast CL reaction of luminol–KIO_4–$K_3Fe(CN)_6$ was observed to be completed in 0.65 s. It was adopted in a simple CE-CL system efficiently to avoid the peak-tailing and overlapping. With this compact system, an indirect determination of rhamnose, D-fructose, sucrose and β-cyclodextrin was realized based on the corresponding negative CL peaks. These peaks were due to the displacement of luminol anions by the analyzed saccharide molecules in alkaline separation electrolyte. In this way, these four saccharides could be separated and determined in 16 min with adequate sensitivities and stabilities. No derivatization or pretreatment was required for the analysis, and it presents an attractive opportunity for routine tests of mono-, di- and oligo-saccharides in a compact CE-CL system, even as a microchip device.

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1. Introduction

Without the interference of stray emission from optical sources, chemiluminescence (CL) is known to be sensitive as an attractive detection method [1]. It has been adopted to determine molecules in some complex samples with separation of capillary electrophoresis (CE) [2,3]. Unfortunately, very few analytes have CL abilities [4] and the CL detection is a time-consuming kinetic process different from other spectrum technologies, requiring careful design of the detection interface to avoid peak-tailing and overlapping [5]. Efforts to overcome these difficulties have been reported, such as an end-column sheath flow interface [6], an end-column detector with fiber optic to transport CL emission [7], and a novel rotary cell [8] for capillary electrophoresis-chemiluminescence detection (CE-CL). However, the continuous consumption of reaction reagents and the complexity of interfaces made these trials unsatisfactory, especially in a CE-CL microchip device [9]. In principle, the simplest interface of CE-CL detection should be only composed of a reaction cell in front of the detector, a photomultiplier tube (PMT) as usual. This efficient end-column CE-CL scheme should be performed with fast CL reactions to avoid overlapping of the CE-CL peaks. The separated species should flow into the cell, lead optical emission and be consumed quickly to avert mixing with CL signals of subsequent analytes.

In this experiment, an ultra-fast CL process of luminol–KIO_4–$K_3Fe(CN)_6$ was recorded to be completed in 0.65 s with strong emission. It provided a favorable opportunity for realizing a simple and efficient end-column CE-CL system, especially while significant negative CL signals were discovered following with the introduction of carbohydrates. In the capillary, an alkaline solution of luminol was used as the separation electrolyte, causing a high CL baseline once it flowed into the reaction cell with electroosmotic flow (EOF). The molecules of saccharides existed as anions similar to those of luminol [10] in this alkaline separation electrolyte, and the displacement of luminol by saccharides led to a decrease in CL intensity. In this way, a novel method of CL detection for carbohydrates coupled with CE was developed by the corresponding negative peaks in electropherograms.

To the best of our knowledge, this is the first report of indirect CE-CL determination for carbohydrates, a difficult-to-detect group with little ultraviolet (UV) absorbance [11] or fluorescence [12]. Though highly sensitive detections of sugars were achieved with electrochemical technologies, the reproducibility was relatively poor because of uncertainty associated with the electrode/capillary alignment during an electrophoresis run and from run to run in addition to the problem of electrode pollution [13]. A novel aerosol chemiluminescent detector coupled to

Abbreviations: CL, chemiluminescence; CE, capillary electrophoresis; CE-CL, capillary electrophoresis-chemiluminescence detection; PMT, photomultiplier tube; EOF, electroosmotic flow; UV, ultraviolet; LOD, detection limit; DMSO, dimethyl sulfoxide; HV, high voltage power supply; PC, personal computer; CE-AD, capillary electrophoresis-amperometric detection; RSD, relative standard deviation.

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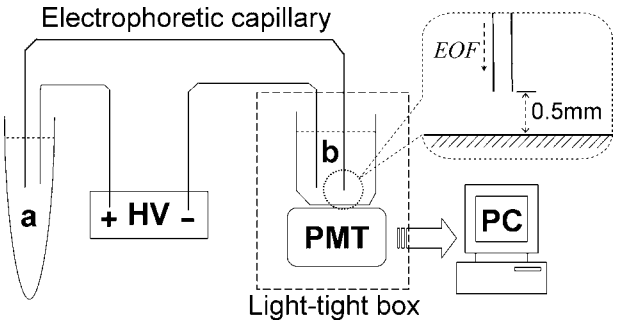

Fig. 1. Schematic diagram of the proposed CE-CL detection system. a, separation electrolyte reservoir; b, CL reaction cell and ground end buffer reservoir; HV, high voltage power supply; PMT, photomultiplier tube; PC, personal computer; EOF, electroosmotic flow.

CE was developed by the group of Zhang for saccharides analysis, but the porous alumina prepared in quartz tube was problematic with unsatisfactory sensitivities for the analyzed sugars [14]. In this investigation, a simple and efficient CE-CL system was realized, relying on an ultra-fast luminol–KIO_4–$K_3Fe(CN)_6$ CL reaction. In this compact system, negative peaks of carbohydrates were discovered and adopted for indirect determination of rhamnose, D-fructose, sucrose and β-cyclodextrin with limits of detection (LOD) between 1.1×10^{-5} and 1.9×10^{-5} mol/L. The validation features were tested, and the mechanism of this method was also studied. This is a novel approach for separation and detection of UV and fluorescence inactive carbohydrates. Furthermore, this method revealed an attractive opportunity for analysis of mono-, di- and oligo-saccharides in a real microchip CE-CL device.

2. Experimental

2.1. Reagents and solutions

Luminol was purchased from Sigma–Aldrich Co. (Saint Louis, USA). Sucrose, β-cyclodextrin, rhamnose and D-fructose were purchased from J&K Scientific Ltd. (Shanghai, China). All other reagents were of the analytical reagent grade and used as received from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The ultrapure water of 18.25 MΩ/cm was produced with an Aquapro AWL-0502-P ultrapure water system (Chongqing, China) and used in experiment.

The luminol solution was prepared daily by dissolving 0.018 g luminol in 10 mL 0.02 mol/L NaOH solution of 10% DMSO (dimethyl sulfoxide). This solution was filtered through a membrane with 0.45 μm pore-size and used as the separation electrolyte in CE. A solution of 0.1 mol/L Na_2CO_3 was prepared, and adjusted to pH 12.5 with 1 mol/L NaOH for dissolving KIO_4 to get a stock solution of 0.01 mol/L KIO_4, which was mixed with $K_3Fe(CN)_6$ and NaOH to gain the CL reaction solution as required. The stock solutions of sucrose (0.1 mol/L), β-cyclodextrin (0.01 mol/L), rhamnose (0.1 mol/L) and D-fructose (0.1 mol/L) were prepared with ultrapure water and diluted in the subsequent operations as needed. All the sample solutions were filtered through a membrane with 0.22 μm pore-size prior to being introduced into the capillary.

2.2. Apparatus and instrumental setup

The CE-CL system used in our experiment was lab-constructed, as shown in Fig. 1. It was composed of two parts: separation and detection. The CE separation was performed with a high voltage power supply (0–30 kV, Shanghai Institute of Applied Physics, Shanghai, China) and a 25 μm i.d. × 65 cm length fused-silica electrophoretic capillary (Yongnian Reafine Chromatography Ltd., Hebei, China). The CL detection was performed by the PMT of an IFFL-D flow-injection luminescent analyzer (Xi'an REMEX Analyse Instrument Co. Ltd., Xi'an, China). A 10 mL quartz beaker, which was filled with the CL reaction solution, was taken as the CL reaction cell and buffer reservoir at the ground end of electrophoretic capillary. The static injection tests were performed in the same CL reaction cell and PMT-computer recording system, without the electrophoretic capillary and high voltage power supply as in Fig. 1.

2.3. Procedure

A series of static injection tests were taken to find the optimized CL detection conditions and discover the mechanism of the negative CL signals forming with the separated saccharides in CE. With a syringe, 20 μL solution of luminol or a mixture of luminol and carbohydrates was injected into the cell and reacted with the CL reaction solutions, while the emission was recorded by the PMT-computer system.

In the CE-CL system, an uncoated capillary was rinsed with 0.1 mol/L NaOH solution for 20 min, following with water for 10 min and the separation electrolyte of luminol for 20 min prior to each operation. The analytical samples were electrokinetically introduced for 10 s at 15 kV and the CE separation was carried out at the same voltage. The CL reaction solution of 2×10^{-3} mol/L KIO_4, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1 mol/L NaOH was filled in the cell to perform efficient end-column CL detection for the separated saccharides.

3. Results and discussion

3.1. Fast CL reaction of luminol–KIO_4–$K_3Fe(CN)_6$

As a kinetic process, CL emission occurs, rises and terminates along with the chemical reaction. So, it is usually a time consuming detection method and great challenges were faced to design an end-column CE-CL system with the avoidance of tailing or overlap of CL peaks [8]. In our previous work, an ultra-fast CL reaction was found to be able to overcome these difficulties quite well [15]. However, as the most frequently used CL reagent, luminol was never studied in this way. Herein, efforts were taken to find fast CL reaction procedures of luminol with strong emission. Three typical CL kinetic curves of the static injection tests are shown in Fig. 2. Firstly, the most frequently used oxidant H_2O_2 was tested to react with luminol in various conditions. As indicated in Fig. 2(A), the duration of CL emission was long even if some catalyst, such as $K_3Fe(CN)_6$ or hemin, was involved in reaction solution due to the slow rate of luminol–H_2O_2 reaction. Though the emission was quite strong, it was incompetent to realize a compact CE-CL system, and more CL reactions of luminol were tested.

As demonstrated in Fig. 2(B), the strong and stable oxidizing agent KIO_4 was discovered to be able to consume luminol much more quickly under the catalysis of $K_3Fe(CN)_6$, with a relatively weak emission. Once the NaOH concentration was increased in this reaction solution of luminol–KIO_4–$K_3Fe(CN)_6$, an ideal CL process was recorded as shown in Fig. 2(C). This ultra-fast CL reaction could be finished just in 0.65 s with strong emission. The NaOH concentration contained in this reaction solution was found to be a key role for such a quick and strong CL process, as demonstrated by further static injection tests shown in Fig. 3. Higher concentrations of NaOH led to higher CL emissions with shorter durations, and 1 mol/L NaOH was adopted in the subsequent experiments.

Various concentrations of KIO_4 and $K_3Fe(CN)_6$ were also tested for this CL reaction. From the data reported in Table 1, a weaker and shorter CL process was observed with more $K_3Fe(CN)_6$, while the amount of KIO_4 did not make a significant difference. Considering

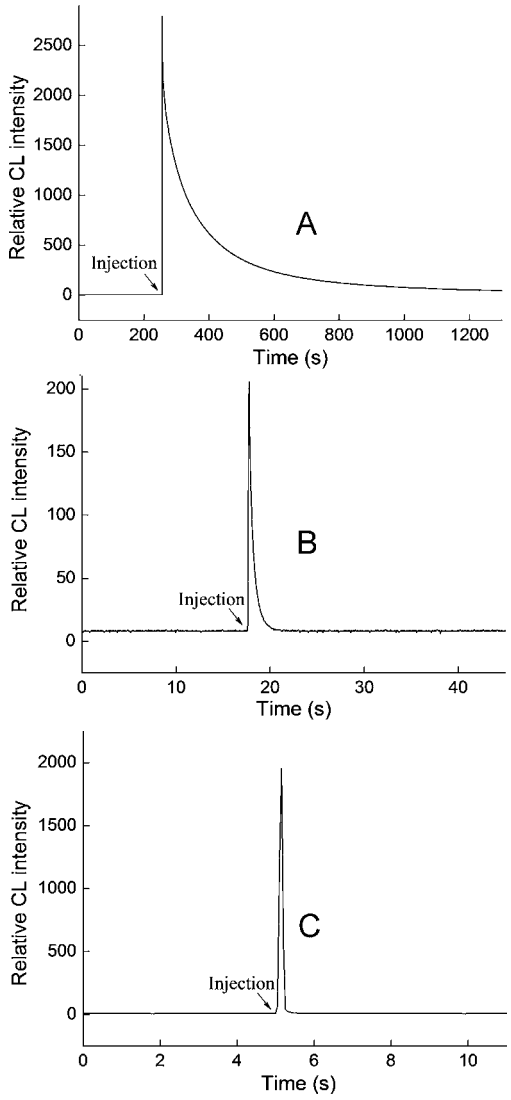

Fig. 2. Three typical kinetic curves for CL reactions of luminol in a series of static injection tests. The experiment was performed with injection of 20 μL 1×10^{-3} mol/L luminol solution into the CL reaction cell, which was filled with (A) 1×10^{-2} mol/L H_2O_2, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1×10^{-2} mol/L NaOH; (B) 2×10^{-3} mol/L KIO_4, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1×10^{-2} mol/L NaOH; (C) 2×10^{-3} mol/L KIO_4, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1 mol/L NaOH. The CL emissions were recorded by the PMT, which was set at −200 V with a sample rate of 20 s^{-1}.

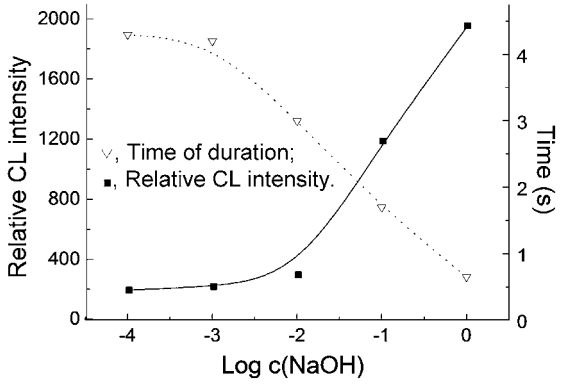

Fig. 3. The effect of NaOH concentration on the luminol–KIO_4–$K_3Fe(CN)_6$ CL reaction system. The results were obtained with the same static injection procedure as in Fig. 2(C), except for the various amount of NaOH in CL reaction solutions.

Table 1
The effect of various amount of KIO_4, $K_3Fe(CN)_6$ on the CL reaction.[a]

$c(KIO_4)$	$c(K_3Fe(CN)_6)$		
	0.001 mol/L	0.01 mol/L	0.1 mol/L
2×10^{-4} mol/L	*I*: 8506 *t*: 2.40 s	*I*: 1235 *t*: 0.45 s	*I*: 62 *t*: 0.30 s
2×10^{-3} mol/L	*I*: 9543 *t*: 2.60 s	*I*: 1956 *t*: 0.65 s	*I*: 83 *t*: 0.20 s
2×10^{-2} mol/L[b]	*I*: 10274 *t*: 1.65 s	*I*: 1181 *t*: 0.75 s	*I*: 79 *t*: 0.25 s

[a] The static injection tests were taken as in Fig. 2 with 1 mol/L NaOH in the reaction solutions; *I*, relative CL intensity; *t*, time of CL duration.
[b] This KIO_4 solution was prepared at 30 °C.

Table 2
The influence of carbohydrates on the CL emission.[a]

Carbohydrate	Relative CL intensity[b]	
	10^{-3} mol/L	10^{-2} mol/L
Sucrose	25,483	27,233
β-Cyclodextrin	25,298	25,225
Rhamnose	24,872	25,051
D-Fructose	24,970	24,421

[a] Experimental procedure: 20 μL solution of luminol (1×10^{-2} mol/L)–saccharides (10^{-3} or 10^{-2} mol/L) was injected into the CL reaction cell, which was filled with 2×10^{-3} mol/L KIO_4, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1 mol/L NaOH, while the emission was recorded by PMT (−200 V, 20 s^{-1}).

the emission intensity and duration, a solution of 2×10^{-3} mol/L KIO_4, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1 mol/L NaOH was filled in the CL reaction cell (b in Fig. 1) to obtain satisfactory results as shown in Fig. 4.

3.2. Indirect CE-CL determination of carbohydrates

As an important biological component in living bodies, carbohydrate caught extensive attention in analytical chemistry [16], and a novel phenomenon of decreased CL signals coming with saccharides was observed in our CE-CL system. The alkaline luminol solution was filled in the capillary as separation electrolyte to form a high and constant CL baseline once the CE started; then saccharide samples were introduced into the capillary with corresponding negative CL peaks appearing while those saccha-

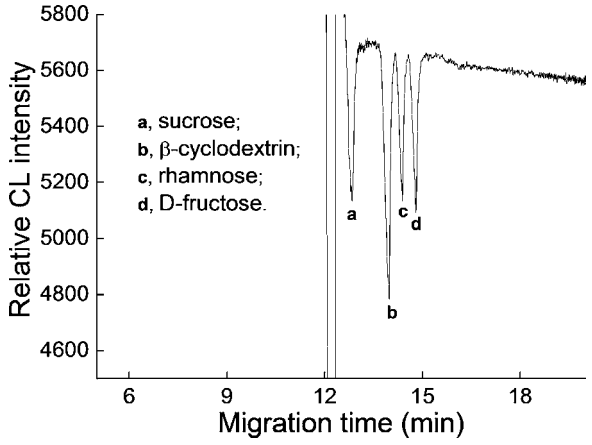

Fig. 4. The indirect CE-CL electropherogram of carbohydrates in our compact CE-CL system. The CE separation conditions: capillary, 25 μm i.d. × 65 cm length; separation electrolyte, 0.01 mol/L luminol dissolved in 0.02 mol/L NaOH solution of 10% DMSO; separation voltage, 15 kV; sample introduction, 15 kV × 10 s. The CL detection conditions: CL reaction solution, 2×10^{-3} mol/L KIO_4, 1×10^{-2} mol/L $K_3Fe(CN)_6$ and 1 mol/L NaOH; detector, PMT was set at −800 V with a sample rate of 20 s^{-1}.

Table 3
The analytical parameters for this proposed CE-CL method.[a]

Parameters	Theoretical plate number	RSD of the migration time[b]	LOD (S/N = 3) (mol/L)	Linear range (mol/L)	Regression equation[c]
Rhamnose	65,700	1.16%	1.9×10^{-5}	6×10^{-5}–1×10^{-2}	$I = 5.25 \times 10^5 c + 1$
D-Fructose	69,000	1.15%	1.8×10^{-5}	5×10^{-5}–1×10^{-2}	$I = 5.56 \times 10^5 c - 8$
Sucrose	29,300	0.96%	1.8×10^{-5}	5×10^{-5}–1×10^{-2}	$I = 5.43 \times 10^5 c - 3$
β-Cyclodextrin	38,400	1.12%	1.1×10^{-5}	3×10^{-5}–1×10^{-2}	$I = 8.82 \times 10^5 c + 7$

[a] The results shown were gained from 6 replicate measurements in the same experimental conditions of Fig. 4.
[b] RSD, the relative standard deviation.
[c] I, the relative CL intensity of negative peaks; c, the concentration of saccharides.

rides flowed into the CL reaction cell by electrophoresis. Based on this observation, four typical saccharides of rhamnose, D-fructose (mono-saccharides), sucrose (di-saccharide) and β-cyclodextrin (oligo-saccharide) were successfully separated and determined in our proposed CE-CL system with demonstration of the electropherogram in Fig. 4.

Similar as our previous investigation of capillary electrophoresis-amperometric detection (CE-AD) for mono-saccharides [17,18], the amount of NaOH contained in the separation electrolyte was found to be critical for this CE-CL carbohydrate analysis. Higher concentration of NaOH led to a better separation and a longer migration time, as indicated by Fig. 5. In our experiment, the baseline separation of those analyzed saccharides was accomplished in 16 min as demonstrated in Fig. 4, while 0.02 mol/L NaOH–luminol solution was filled in the capillary.

3.3. Mechanism of our proposed CE-CL method

In the previously reported indirect CE-CL methods for the determination of monoamines, catechol [19], catecholamines [20] and amino acids [21], Cu(II)-catalyzed luminol CL reaction was adopted to gain a high and constant background emission. Since these analytes could form stable complexes with Cu(II), inverted analyte peaks were recorded due to the decreased catalytic activity of Cu(II)–analyte complexes. Obviously, this explanation could not be applied for the negative peaks of saccharides formed in this method.

More static injection tests, as described in Table 2, were performed to figure out the reason. No CL inhibitive effect was found with the injection of saccharides in our luminol–KIO_4–$K_3Fe(CN)_6$ reaction system. In fact, the CL intensities resulting from injection of luminol–saccharides were even greater than those of luminol. This is consistent with Yamada's observations for CL reactions between periodate and polyhydroxyl compounds [22].

As suggested by the literature [23], charge displacement of ions in the electrophoresis could be another reason for the appearing of negative CL peaks. Connecting with the observations in our experiment, we tended to attribute this proposed indirect CE-CL method to be the result of ions displacement as described below. In alkaline separation electrolytes, the molecules of luminol were negatively charged and replaced by the anions of saccharides with electrophoresis. As a result, less luminol flowed out of the capillary together with the analyzed carbohydrates, causing inverted CL peaks in corresponding CE-CL electropherograms.

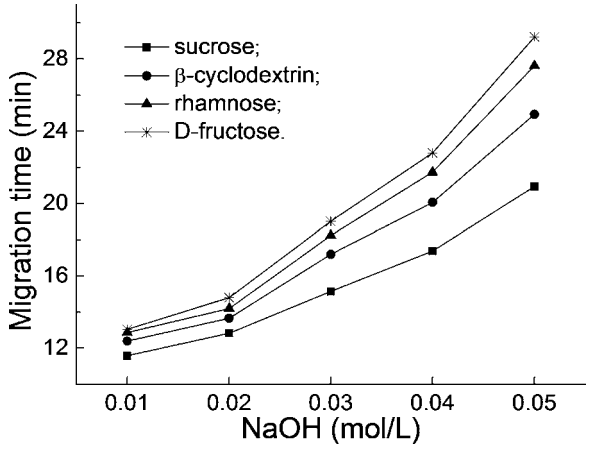

Fig. 5. The effect of NaOH concentration on migration time of rhamnose, D-fructose, sucrose and β-cyclodextrin with CE-CL conditions same as in Fig. 4.

3.4. Validation and quantification features

As a newly developed detection method, some important features such as the separation efficiency, sensitivity and reproducibility were measured. Briefly, these determinations were performed in the same conditions as those of Fig. 4 with proper concentration solutions of the analyzed saccharides. As demonstrated by the results in Table 3, this proposed CE-CL method exhibited adequate theoretical plate numbers (29,300–69,000), low limit of detection (LOD: 1.1–1.9 × 10^{-5} mol/L, S/N = 3) and good reproducibility of migration time (RSD: 0.96–1.16%). The present achievement of detecting mono-, di- and oligo-saccharides revealed an exciting application possibility of this work.

4. Conclusions

In this experiment, an efficient CE-CL system was set up with a simple interface based on a newly recorded ultra-fast CL reaction of luminol–KIO_4–$K_3Fe(CN)_6$. With this compact scheme, negative CL peaks of mono-, di- and oligo-saccharides were discovered due to the displacement of luminol anions in the alkaline separation electrolyte. Based on these observations, we firstly explored an indirect CE-CL detection method of carbohydrates. Without derivatization or pre-treatment, four typical saccharides of rhamnose, D-fructose, sucrose and β-cyclodextrin were determined with adequate sensitivity and reliability in this compact CE-CL system. The method developed in this paper provides an alternative approach for the routine analysis of carbohydrates, and reveals attractive opportunity for realizing a simplified CE-CL system.

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References

[1] Y. Weizmann, Z. Cheglakov, I. Willner, J. Am. Chem. Soc. 130 (2008) 17224–17225.
[2] J. Wang, W. Huang, Y. Liu, J. Cheng, J. Yang, Anal. Chem. 76 (2004) 5393–5398.
[3] X. Ji, Z. He, X. Ai, H. Yang, C. Xu, Talanta 70 (2006) 353–357.
[4] M. Yamaguchi, H. Yoshida, H. Nohta, J. Chromatogr. A 950 (2002) 1–19.
[5] X. Huang, Z. Fang, Anal. Chim. Acta 414 (2000) 1–14.
[6] K. Tsukagoshi, A. Tanaka, R. Nakajima, T. Hara, Anal. Sci. 12 (1996) 525–528.
[7] R. Dadoo, A.G. Seto, L.A. Colon, R.N. Zare, Anal. Chem. 66 (1994) 303–306.
[8] J. Wang, L. Li, W. Huang, J. Cheng, Anal. Chem. 82 (2010) 5380–5383.
[9] X. Huang, J. Ren, Electrophoresis 26 (2005) 3595–3601.

[10] E.H. White, O. Zafjriou, H.H. Kagi, J.H.M. Hill, J. Am. Chem. Soc. 86 (1964) 940–941.
[11] Y. Mechref, G.K. Ostrander, Z.E. Rassi, J. Chromatogr. A 695 (1995) 83–95.
[12] R.A. Evangelista, M. Liu, F.A. Chen, Anal. Chem. 67 (1995) 2239–2245.
[13] C. Patrick, Capillary Electrophoresis Theory And Practice, 2nd ed., CRC Press LLC, FL, 1998.
[14] G. Huang, Y. Lv, S. Zhang, C. Yang, X. Zhang, Anal. Chem. 77 (2005) 7356–7365.
[15] J. Zhu, L. Shu, F. Zhang, Z. Li, Q. Wang, P. He, Y. Fang, Luminescence, in press, http://onlinelibrary.wiley.com/doi/10.1002/bio.1379/pdf.
[16] Q. Wang, Y. Fang, J. Chromatogr. B 812 (2004) 309–324.
[17] Q. Hu, T. Zhou, L. Zhang, Y. Fang, Analyst 126 (2001) 298–301.
[18] S. Dong, S. Zhang, X. Cheng, P. He, Q. Wang, Y. Fang, J. Chromatogr. A 1161 (2007) 327–333.
[19] H. Tsai, C. Whang, Electrophoresis 20 (1999) 2533–2538.
[20] Y. Zhang, B. Huang, J. Cheng, Anal. Chim. Acta 363 (1998) 157–163.
[21] S. Liao, C. Whang, J. Chromatogr. A 736 (1996) 247–254.
[22] J. Lin, M. Yamada, Anal. Chem. 71 (1999) 1760–1766.
[23] J. Ren, X. Huang, Anal. Chem. 73 (2001) 2663–2668.