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Journal of Chromatography A, 844 (1999) 341–348

JOURNAL OF CHROMATOGRAPHY A

On-line chemiluminescence detection for capillary electrophoresis based on the reaction of barium peroxide with luminescence reagents

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Received 19 October 1998; received in revised form 17 February 1999; accepted 23 February 1999

Abstract

On addition of trace amounts of luminol or lucigenin to the milligram amounts of barium peroxide (BaO_2) solid powder, strong chemiluminescent phenomena have been observed. Based on this observation, a novel on-line solid-phase chemiluminescence detector was designed for capillary electrophoresis. A 27 cm×75-μm I.D. column with a 0.3-cm length detection window was used. A small amount of BaO_2 powder was packed into the detection window section. After the separation of luminol, lucigenin and *N*-(4-aminobutyl)-*N*-ethylisoluminol (ABEI), the analytes may be detected on-line. The optimum conditions for the capillary electrophoresis separation and detection were investigated. The detection limits were 5×10^{-8}, 7×10^{-8} and 1×10^{-8} mol/l for lucigenin, ABEI and luminol, respectively. The proposed method was also used for the separation and detection of two amino acids labeled with ABEI. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Chemiluminescence detection; Detection, electrophoresis; Amino acids; Aminobutylethylisoluminol; Barium peroxide; Luminol; Lucigenin

1. Introduction

Modem capillary electrophoresis (CE) demonstrated that it is a powerful tool in chemical [1], biomedical and pharmaceutical separations [2–4] since it was firstly introduced by Miller et al. [5] in 1979 and Jorgenson and Lukacs in 1981 [6]. The advantages of high resolution, short analytical time, small sample needed and almost no environmental pollution encouraged a lot of analytical chemists to study in this field. However, in order to eliminate convection, capillaries used in practice were less than 0.1 mm I.D. Due to the use of tiny column in CE, the samples of low concentration were difficult to be detected after the separation. Therefore, the ultra-small samples pose a severe challenge to detector design. In recent years, various methods, including mass spectrometry [7], fluorescence [8], electrochemical [9,10] and chemiluminescence (CL) [11–13] detection have been reported. Particularly, optical detectors for CE have been systematically reviewed [14], and a review of CE–CL detection was also reported [15]. In these detection methods, CL can be anticipated to provide high sensitivity because of its low background nature. Most CL systems have detection limits of lower than 10^{-10} mol/l [16,17]. Combination of the high separation

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PII: S0021-9673(99)00271-X

ability of CE with high sensitive of CL is an attractive research brand. About 40 papers [15,18] concerning with CE–CL detection have been reported since this method firstly proposed by Dadoo et al. [19] in 1992.

The high sensitivity of CL is well known, but that many CL reactions need CL reagent, catalyst and basic medium is also well-known. Therefore, almost all of the CL systems used in CE required a detection interface or a reagent mixing tee [15,18]. The interface connection or mixing tee made the detector complicated, and it is one of the main results of peak broadening. The slow CL reaction kinetics in the flow system is also a reason for the low number of theoretical plates. Recently, the basic metal peroxides were evaluated as CL reactions in our laboratory [20]. Strong CL was observed solely on adding luminol, ABEI or lucigenin solution to BaO_2 or MgO_2 powders. The CL characteristics of this heterogeneous reaction system were different from those of homogeneous reaction systems [21]. This new CL system was applied in the present work as an on-line CE detector for the determination of luminol, lucigenin and ABEI. Sharp peaks and highly sensitive results were obtained.

2. Experimental

2.1. Chemicals and solutions

Luminol, bis(*N*-methylacridinium) nitrate (lucigenin) and *N*-(4-aminobutyl)-*N*- ethylisoluminol (ABEI) were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Barium peroxide (particle size smaller than 5 μm, Nacalai Tesque, Kyoto, Japan) was of guaranteed reagent grade. Arginine, glycine and di(*N*-succinimidyl) carbonate (DSC) were also purchased from Tokyo Kasei. Both tris(hydroxymethyl)aminomethane (Tris) and boric acid (guaranteed reagent grade) were products of Kanto (Tokyo, Japan). The stock solutions of 1.0×10^{-4} mol/l lucigenin, luminol and ABEI were prepared in a 0.05 mol/l borate buffer solution (pH 8.3). Each dilution reagent solution was prepared daily from the stock solution. The water used was prepared by deionization of distilled water from Milli-XQ (Millipore). All of chemicals were of analytical grade and used as received.

2.2. Apparatus

The CE system assembled from a R585 photomultiplier tube (Hamamatu Photonics, Shizuoka, Japan), a HCZE-30 PN0.25 high-voltage power supply (Matsusada Precision Devices, Japan) and a C-R6A Chromatopac Recorder (Shimadzu, Japan). Separations were carried out in 0.075 mm I.D.× 0.375 mm O.D. fused-silica capillaries (GL Science, Tokyo). The total length of the capillary was 27 cm, effective length was 25 cm. The detection section was 0.3 cm in length. BaO_2 powder was packed in this section. Sample introduction was performed by the electroinjection method at a constant voltage (200 V/cm) for a fixed period (5~10 s). The output from the photomultipliter tube (operated at −800 V) was fed to a TR8641 Electronic Picoammeter (Advantest, Tokyo, Japan) connected to the recorder to produce electropherograms. The CL batch method was carried out with a LumiCounter 600 (Microtec NITI-ON, Funabashi, Japan).

2.3. Methods

Before filling BaO_2 particles, the capillary was rinsed with 0.1 mol/l NaOH for 10 min and then flushed with distilled water–ethanol (1:1, v/v) for ca. 10 min. Then, the capillary was dried in an air stream for ca 1 h. The preparation of CL detector in the capillary is shown in Fig. 1. In order to avoid the BaO_2 filling into the separation section of the capillary, a 0.05-mm O.D. platinum wire was inserted into the capillary until the detection window. By rubbing up and down the other side of the capillary onto BaO_2 powder loaded on a glass slide (Fig. 1a), BaO_2 powder was slowly and carefully put into the detection window section. Finally, the buffer solution was placed into the capillary using a 0.5-ml syringe (Fig. 1b).

The labeled amino acids with ABEI was carried out according to the methods of Kawasaki et al. [22] and Dadoo et al. [23]. First, a 5 mmol/l solution of ABEI in methanol was added to an equal volume of

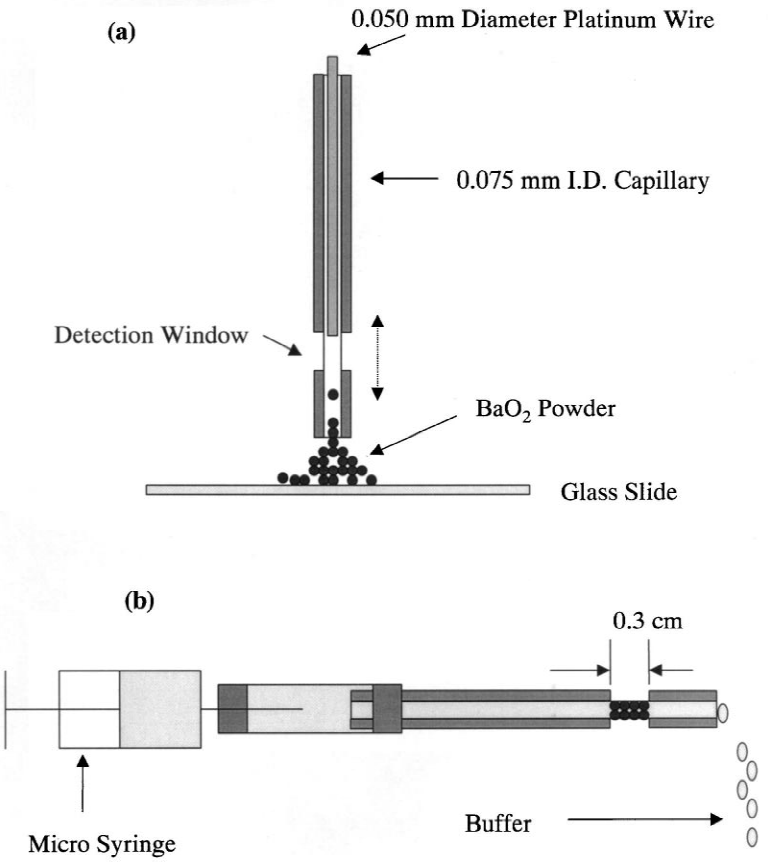

Fig. 1. Schematic representation of the preparation procedure for the on-line CE–CL detection window. (a) Filling BaO_2 powder into capillary; (b) carefully and slowly injecting buffer solution into the capillary using a microsyringe.

a 5 mmol/l solution of DSC in acetonitrile. The resulting solution was sonicated for 2 h. Then, the desired amino acids were added (in excess) to aliquots of the ABEI–DSC solution, and the aliquots were also sonicated for ca 2 h. The labeled amino acid solutions were diluted by a factor of 10 or more in the separation buffer before injection.

The batch experiments for the CL reaction were described in our previous work [20]. A total of 20 μl of analyte solution were injected into the reaction vessel (5.0 mm I.D., 0.8 ml volume), which was loaded with 10 mg of dried BaO_2 powder in the bottom. The peak height of the signal recorded was measured as a CL signal.

3. Results and discussion

3.1. Batch experimental

In our previous works [20,21], we investigated the CL reactions of BaO_2 with luminol and lucigenin. The CL signals shown in Fig. 2 indicated that the oxidation reactions of luminol, ABEI or lucigenin with BaO_2 were relatively fast. Without addition of any other oxidants or bases, the CL signals from the injections of luminol, lucigenin and ABEI into BaO_2 responded to the detection limits of 3×10^{-12} mol/l luminol, 5×10^{-12} mol/l lucigenin and 1×10^{-11} mol/l ABEI, respectively. The CL mechanism was

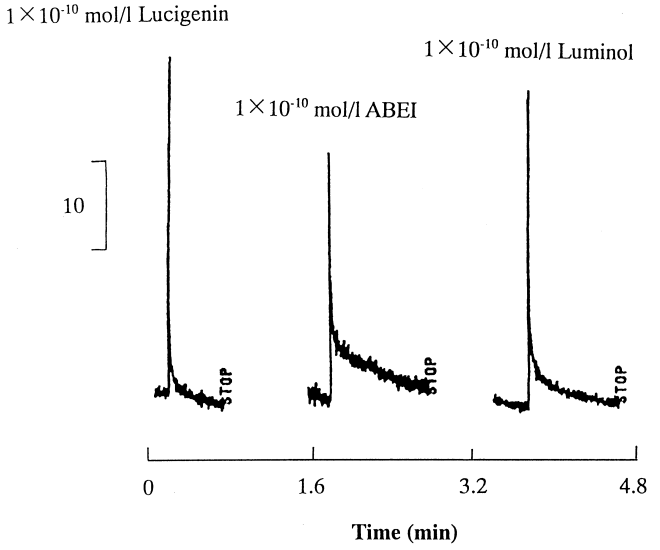

Fig. 2. The CL signals of the reactions of BaO_2 with lucigenin, ABEI and luminol with the batch method. Conditions: 20 μl of 1×10^{-10} mol/l sample (pH 8.3) were injected into 0.010 g of BaO_2.

considered to be the reactive oxygen species ($O_2^{\cdot-}$) existed on the surface of BaO_2 particles [21]. Therefore, the environmental media of BaO_2 particles influenced the CL intensity significantly. The investigations of optimal conditions, including the buffer concentration, pH and organic solvent, are important for both separation and detection.

3.2. Preparation of on-line CE–CL detection window

As shown in Fig. 1, the preparation of an on-line CE–CL detector should be finished with two steps, packing the BaO_2 powder into the detection window section and filling the electrolyte solution into the capillary. Considering the inner diameter of the capillary, particles which are too large are difficult to pack. We selected particle size of BaO_2 powder smaller than 5 μm for our experiment. In order to avoid the BaO_2 powder filling the separation section of the capillary, a 50-μm O.D. platinum wire was inserted into the capillary up to the detection winder. Then, as demonstrated in Fig. 1a, the BaO_2 powder loaded on a glass slide was slowly and carefully placed into another shorter side of the capillary by rubbing the capillary up and down. After a 0.40-cm length of the capillary was packed with BaO_2 powder, it was slowly pushed into the detection window using another 50-μm O.D. platinum wire. This packed part could be closed to 0.30 cm. Too much BaO_2 power packed into the window section will cause difficulty in filling the buffer into the capillary. If BaO_2 power is loosely packed, some particles will be lost during column flushing and electrophoresis. With our present packing method, there were no serious differences between the two capillaries for both the separations and CL intensities, under the same experimental conditions. We also found that it was unnecessary to use any frit for the protection of the BaO_2 particles entering into the separation capillary, because the directions of electroosmotic flow and electrophoresis are the same. The BaO_2 particles were impossible to move opposite to the direction of electroosmotic flow, even though there were very small amounts of particles scattered into the shorter section of the capillary.

After successfully packing the BaO_2 powder into the detection window, the buffer solution was filled into the capillary using a microsyringe. The buffer can only be filled from the left end of the capillary (see Fig. 2) to right side (short section) because the left section of the detection window is for separation. In order to avoid the BaO_2 particles moving from the detection window, the flow-rate of the buffer solu-

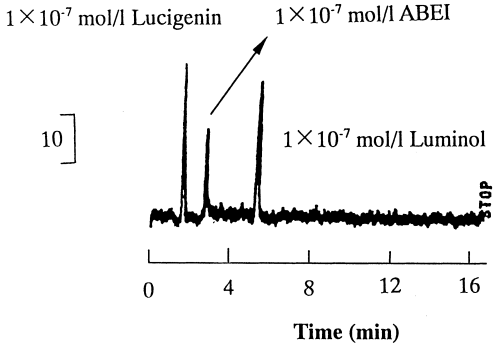

Fig. 3. Electropherogram of the separation and on-line CE–CL detection of lucigenin, ABEI and luminol. Conditions for CE–CL detection: 0.05 mol/l, pH 8.3, borate buffer containing 5% acetonitrile. The total length of the capillary was 27 cm, effective length was 25 cm. Sample introduction was performed by electroinjection at 200 V/cm for 5 s.

tion must be controlled at lower than 1 cm/mm: both the BaO_2 powder packing and buffer filling must be carried out very carefully.

3.3. Buffer solution

Three different buffer solutions, phosphate, sodium borate and Tris–H_3BO_3 have been compared. At the same concentration (0.05 mol/l, pH 8.0), the CL intensities were almost the same. But the separation efficiency of lucigenin, ABEI and luminol are influenced by electrolyte. An amount of 0.05 mol/l phosphate caused a high current and peak broadening. Tris–H_3BO_3 was suitable for the separation but it made the lifetime of the detector shorter. This may be due to the slow reaction of BaO_2 with Tris. Using sodium borate buffer (pH 8.0), we found that both separation and CL intensity were better than with the other two buffers. At pH 8.3, both CL signals from batch and CE methods are shown in Fig. 2 and Fig. 3, respectively. The shape signals are due to the fast reaction of BaO_2 with analytes. The concentration of buffer solution influenced not only the separation but also the CL intensity. From Fig. 4, with increasing buffer concentration, the theoretical plate number of the separation increased up to 0.05 mol/l borate. Then the CE efficiency decreases with even higher borate concentrations, which may be due to the changes of the salt concentration of the buffer solution. We also think that the significant CE efficiency loss may be caused by the effect of 'power cell' on the electroosmotic flow (EOF) when the

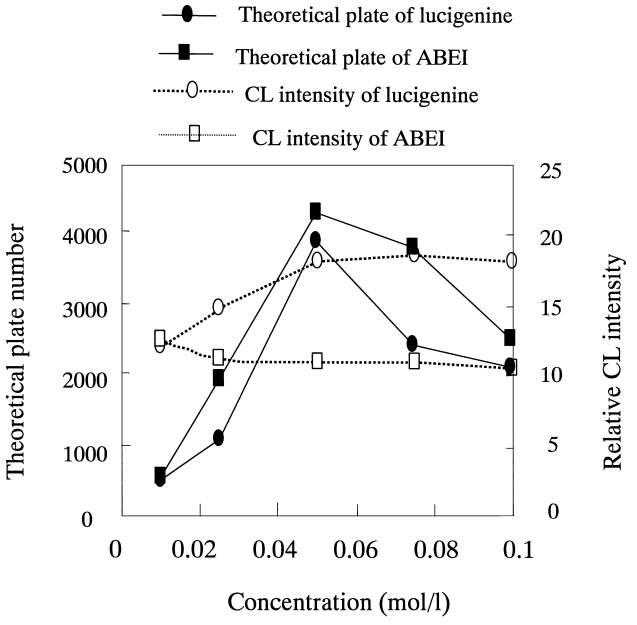

Fig. 4. Effect of concentration of buffer solution on the separation and CL intensity. Both concentrations of ABEI and lucigenin were 1×10^{-7} mol/l. Other conditions are the same as in Fig. 3, except for the buffer concentration.

buffer concentration is increased. The CL intensities of ABEI and lucigenin are constant in the range of 0.05 to 0.1 mol/l buffer. Of course, in unbuffered solution the separation cannot be in progress. Considering both separation and CL reaction, a pH 8.3 of 0.05 mol/l sodium borate was accepted as one of our separation conditions.

3.4. Effect of buffer pH

In acidic, neutral and basic solution, the activity of BaO_2 is very different. Generally, BaO_2 decomposes to hydrogen peroxide and oxygen in acidic solution or hot water [24]. In neutral or weekly basic solution, BaO_2 is relative stable: there is no gaseous bubble observed on the surface of BaO_2 particles when BaO_2 exists in pH 6–9 borate buffer. The CL intensities of the reactions of luminol, ABEI and lucigenin in the range of pH 3.0–9.0 have been studied by the batch method [20,21]. The strongest CL was not observed at the lower pH range; this result may be due to the decomposition of BaO_2 in acidic medium before the injection of analyte into the cell. The brightest emission has been recorded for pH 8.0–8.5. In order to check the lifetime of BaO_2 oxidation in weekly basic buffer solution, after standing for 2 h, injection of another 20 μl of ABEI solution into the residual BaO_2 was carried out; a CL signal similar to the first one was also observed. The oxidation activity of BaO_2 in pH 8.3 buffer can be maintained for about 3 days. The CL intensities and the separation results were almost the same for 30 injections of the mixture solution of 1×10^{-7} mol/l ABEI, luminol and lucigenin, using a new CE–CL detector. The relative standard derivatives of the CL peak height were in the range of 2.3–5.2%. However, the lifetime of the BaO_2 powder-filled detector is relatively short. It is recommended that the CE–CL detector be prepared daily. On the other hand, at pH 8.0–8.5, the mixture of ABEI, luminol and lucigenin was also separated very well. The acceptable value of pH 8.3 for both separation and CL detection was used.

3.5. Effect of organic solvents on separation and detection

For both separation and detection, the addition of organic solvent to the buffer solution caused a better result. As shown in Table 1, the CL intensity was enhanced by the addition of methanol, ethanol, acetonitrile or tetrahydrofuran (THF). This phenomenon can be explained in terms of the different solubility of the reaction products in water. The CL spectra indicated that the maximum wavelengths of the CL from the reactions of BaO_2 with luminol and lucigenin were 427 and 523 nm, respectively. These wavelengths are the same as the results for luminol and lucigenin reacting with hydrogen peroxide in basic solution. The final products of the CL reactions of lucigenin, as well as luminol, *N*-methylacridone and 3-aminophthalic acid, are difficult to dissolve in water. They are absorbed on the surface of BaO_2 particles, which causes the lowering of the reaction activity of BaO_2. Addition of a proper amount of organic solvent into the buffer is useful for dissolving the CL reaction products. Based on the results of Table 1, acetonitrile was used as part of the reaction medium and CE electrolyte. On the other hand, the separation of the samples was also affected by the organic solvent. Although the mixture of luminol, ABEI and lucigenin can be separated well with

Table 1
Effect of organic solvent on CL intensity and migration time[a]

Solvent	Relative CL intensity			Migration time (min)		
	Lucigenin	ABEI	Luminol	Lucigenin	ABEI	Luminol
Only buffer	5	3	4	1.57	2.84	5.47
Methanol	12.5	9	14	2.20	3.42	6.21
Ethanol	11.0	10	13	2.73	4.21	7.63
Acetonitrile	17.5	11	27	2.11	3.23	5.64
THF	13	8	12	2.50	4.05	6.72

[a] Buffer: 0.05 mol/l H_3BO_3 solution adjusted to pH 8.3 using 0.1 mol/l NaOH solution. The concentration of organic solvents was 10% (v/v). The concentrations of lucigenine, ABEI and luminol were 2×10^{-7} mol/l.

0–10% (v/v) acetonitrile in buffer, 5% of acetonitrile was selected as one of conditions for the separation and detection.

The calibration graphs for the determinations of luminol, lucigenin and ABEI, under the above conditions, are shown in Fig. 5. The CL intensities are dependent on the concentrations of analytes. The detection limits for sole injection of luminol, ABEI and lucigenin were 1×10^{-8}, 7×10^{-8} and 5×10^{-8} mol/l, respectively. These results correspond to approximately 100 amol per injection level.

3.6. Separation of amino acid-labeled with ABEI

ABEI, one of the derivatives of isoluminol, has been widely used as CL pre-label in commercial immunoassays [25,26]. In this work, as an example of the practical applications, two amino acids, arginine and glycine, were labeled with ABEI, separated and detected by the proposed method. The result of separation and the detection of the labeling samples is shown in Fig. 6; five peaks appear in the chart. Peaks 2 and 4 correspond to the CL of arginine and glycine labeled with ABEI. Another two peaks, peaks 1 and 3 are the signals of ABEI and ABEI-DSC. A small peak between peaks 1 and

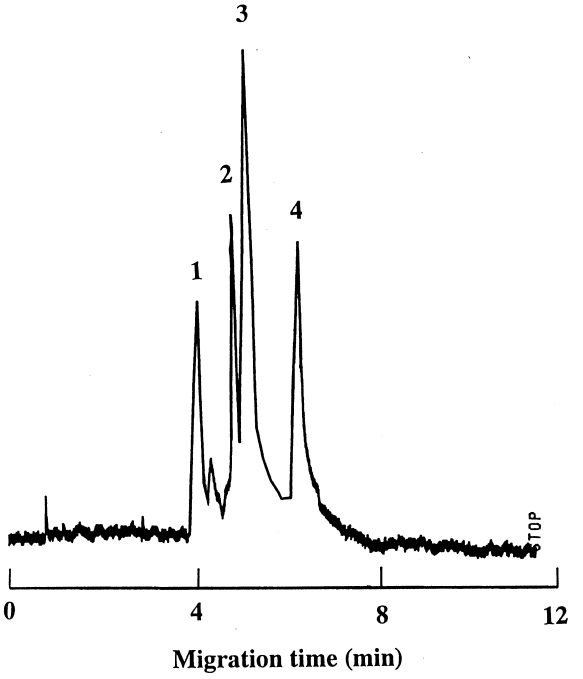

Fig. 6. Electropherogram of arginine and glycines labeled with ABEI. Peaks 1–4 correspond to ABEI, arginine, ABEI-DSC and glycine, respectively. Separation and detection conditions are the same as in Fig. 3.

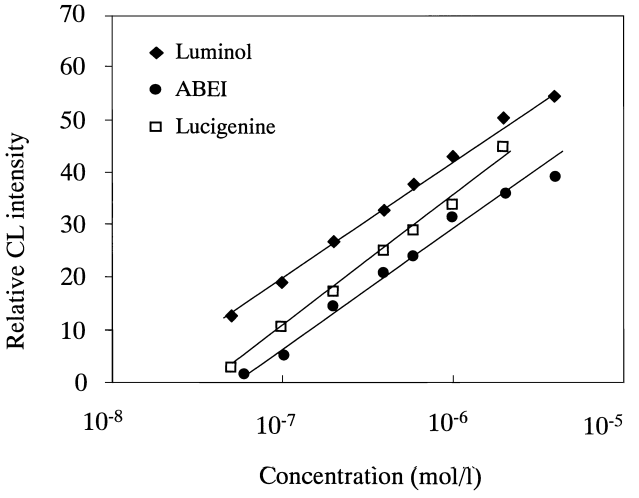

Fig. 5. Calibration graphs from on-line CE–CL detection. Electroinjection time was 5 s at 200 V/cm. Other conditions are the same as in Fig. 3.

2 may correspond to the impure substances. The higher concentrations of the unreacted ABEI and ABEI-DSC, higher peak heights of 1 and 3, can be recorded. It is necessary to point out that too high concentration of ABEI and DSC will influence the separation of samples, although they are beneficial to the labeling. We can clearly see from Fig. 6, that peak 2 is very close to peak 3. Too high concentration of ABEI-DSC will cause peak 2 to be covered by peak 3. So, in this work, the additions of the amount of ABEI and DSC for the labeling process were controlled carefully.

Acknowledgements

This work was partially supported by a grant-in-aid for scientific research (No. 09650894) from the Ministry of Education, Science and Culture of Japan, which we gratefully acknowledge.

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