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Analysis of amino acids and biogenic amines in breast cancer cells by capillary electrophoresis using polymer solutions containing sodium dodecyl sulfate

Yu-Yen Kao [a], Kung-Tien Liu [b], Ming-Feng Huang [a], Tai-Chia Chiu [c,*], Huan-Tsung Chang [a,**]

[a] Department of Chemistry, National Taiwan University, 1 Section 4, Roosevelt Road, Taipei 106, Taiwan
[b] Chemical Analysis Division, Institute of Nuclear Energy Research, Atomic Energy Council, Taoyuan, Taiwan
[c] Department of Applied Science, National Taitung University, 684 Section 1, Chunghua Road, Taitung 95002, Taiwan

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ABSTRACT

We describe simultaneous analysis of naphthalene-2,3-dicarboxaldehyde (NDA)-amino acid and NDA-biogenic amine derivatives by CE in conjunction with light-emitting diode-induced fluorescence detection using poly(ethylene oxide) (PEO) solutions containing sodium dodecyl sulfate (SDS). After sample injection, via EOF 0.1% PEO prepared in 100 mM TB solution (pH 9.0) containing 30 mM SDS entered a capillary filled with 0.5 M TB solution (pH 10.2) containing 40 mM SDS. Under this condition, 14 NDA-amino acid and NDA-amine derivatives were separated within 16 min, with high efficiency ($(1.0–3.2) \times 10^5$ theoretical plates) and sensitivity (LODs at S/N = 3 ranging from 2.06 to 19.17 nM). In the presence of SDS and PEO, analytes adsorption on the capillary wall was suppressed, leading to high efficiency and reproducibility. The intraday analysis RSD values ($n = 3$) of the mobilities for the analytes are less than 0.52%. We have validated the practicality of this approach by quantitative determination of 9 amino acids in breast cancer cells (MCF-7) and 10 amino acids in normal epithelial cells (H184B5F5/M10). The concentrations of Tau and Gln in the MCF-7 cells were different than those in the H184B5F5/M10 cells, respectively. Our results show the potential of this approach for cancer study.

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1. Introduction

Amino acids and biogenic amines play various important roles in many biological systems. For example, amino acids are not only the unit of proteins but also important message transporters, neurotransmitters, and source of energy [1,2]. During the proliferation and migration of cancer cells, amino acids such as alanine (Ala), aspartate (Asp), glutamine (Gln), and glutamate (Glu) act as energy suppliers [3]. Biogenic amines, including dopamine (DA), norepinephrine (NE), and serotonin (5-HT) are metabolites of amino acids and are important neurotransmitters in central and peripheral nervous systems [4]. Because of their important biological roles, many of amino acids and amines are biomarkers for various diseases such as cancers, pheochromocytoma, and neuroblastoma. The concentrations of Gln, serine (Ser), and valine (Val) in lung cancer and normal cells are different [5] and the concentrations of NE and DA in urine specimens above 80 and 400 μg per 24 h, respectively, have been reported to be correlated with pheochromocytoma [6].

In order to determine the concentrations of amino acids and biogenic amines from complicated biological samples like cancer cells, techniques providing high resolution and sensitivity are highly demanded. Capillary electrophoresis (CE) has proved powerful for the separation of complicated biological samples, with advantages of high separation efficiency, short analysis time, and minimum amounts of reagents and sample required [7–9]. Among various CE modes, micellar electrokinetic chromatography using surfactants such as sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide have become especially viable and popular ways for the analysis of biological and environmental samples containing a broad range of compounds including amino acids and amines [10–14].

CE in conjunction with laser-induced fluorescence or light-emitting diode-induced fluorescence (LEDIF) detection provides great sensitivity for amino acids and biogenic amines [15–19]. However, besides three aromatic amino acids, most amino acids are not fluorescent in nature. Thus derivatization of amino acids with reagents such as naphthalene-2,3-dicarboxaldehyde (NDA), fluorescein isothiocyanate, and 3-(2-furoyl)quinoline-2-carboaldehyde to form stable and highly fluorescent derivatives is required when fluorescence detection is applied [19–21]. A

* Corresponding author. Tel.: +886 89 318855x3801; fax: +886 89 342539.
** Corresponding author. Tel.: +886 2 33661171; fax: +886 2 33661171.
E-mail addresses: tcchiu@nttu.edu.tw (T.-C. Chiu), changht@ntu.edu.tw (H.-T. Chang).

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CE-LEDIF system using a low-cost and stable LED as the light source was applied to the determination of amino acids that had been derivatized with NDA [15]. NDA-amino acid derivatives emit light centered at the wavelength of 490 nm when excited at the wavelength of 420 nm.

We have demonstrated that CE using poly(ethylene oxide) (PEO) solution is useful for the separation of biomolecules under discontinuous conditions [15–19,22–24]. In order to provide reproducible and high EOF, TB solutions used to fill a capillary and to prepare PEO solutions are important. Use of high concentrations of TB solution containing SDS minimized adsorption of PEO molecules onto the capillary wall, leading to improved efficiency and sensitivity for the analysis amino acids. Over the TB concentration range (0.4–2.5 M) and pH range (7.0–10.0), we obtained high and reproducible EOF when TB solution (1.5 M, pH 10.0) was used to fill the capillary, mainly due to minimum PEO adsorption onto the capillary wall. Regarding TB solution used to prepare PEO solutions, over the TB concentration range (50–400 mM) and pH range (7.0–9.0), TB solution (200 mM, pH 9.0) provided highest efficiency and reproducibility. When low TB concentrations were used, PEO adsorption onto the capillary wall was greater, leading to low and irreproducible EOF. On the other hand, when high concentrations of TB solutions or TB at low pH values (need more borate) were used, band broadening and irreproducibility were problematic, mainly due to Joule heating. TB solution at pH values higher than 9.0 was not proper because of instability of PEO solution (due to hydrolysis). In this study, we further evaluated parameters such as SDS and PEO concentrations with respect to the separation resolution and efficiency of the CE-LEDIF approach. The practicality of the proposed CE-LEDIF approach was validated by the determination of amino acids and biogenic amines in breast cancer cells (MCF-7) and human epithelial cells (H184B5F5/M10).

2. Experimental

2.1. Chemicals

Dopamine (DA), sodium tetraborate, and tyrosine (Tyr) were obtained from Acros Organic (Geel, Belgium). Alanine (Ala), aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln), glycine (Gly), histidine (His), methanol, NE, serine (Ser), sodium bicarbonate, sodium cyanide (NaCN), SDS, taurine (Tau), tryptophan (Trp), and Val were obtained from Sigma (St. Louis, MO, USA). PEO (Mr 8.0×10^6 g mol^{-1}) was obtained from Aldrich (Milwaukee, WI, USA). NDA and 5-HT were obtained from Tokyo Chemical Industry (Tokyo, Japan). Potassium chloride, potassium dihydrogen phosphate, sodium chloride, sodium phosphate dibasic, and tris(hydroxymethyl)aminomethane (Tris) were products from J.T. Baker (Phillipsburg, NJ, USA). Boric acid was purchased from RiedeldeHaën (Buchs, Switzerland). The stock solution of 400 mM TB was prepared by dissolving 24.23 g of Tris in 500 mL aqueous solution that was adjusted with suitable amounts of boric acid to pH 9.0. Unless otherwise noted, the molarity of Tris represents that of TB buffer. PEO (0.005–0.10 g) was gradually added to each of prepared 100 mM TB solutions (pH 9.0; 50 mL). During the addition of PEO, a magnetic stirring rod was used to produce a well-homogeneous suspension. After the addition was completed, the solutions were stirred for at least 8 h. Prior to use for CE separation, the solutions were degassed with a vacuum system in an ultrasonic tank.

2.2. Apparatus

The basic design of the CE-LEDIF system was described previously [15]. Briefly, a high-voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA) was used to drive electrophoresis. The entire system was placed in a black box possessing a high-voltage interlock. For safety, the high-voltage end of the separation system was placed in a laboratory-made Plexiglas box. An LED (InGaN; maximum output at 405 nm over the range 390–420 nm), obtained from Kwang-Hwa Electronic Material (Taichung, Taiwan), was used for excitation. The light was collected with a 10× objective (numerical aperture = 0.25). One GG 495 cut-off filter was used to block scattered light before the emitted light reached the photomultiplier tube (Hamamatsu R928). The amplified currents were transferred directly through a 10-kΩ resistor to a 24-bit A/D interface at 10 Hz (Borwin, JMBS Developments, Le Fontanil, France) and stored in a personal computer. Fused-silica capillary with 75 μm I.D. and 365 μm O.D. was purchased from Polymicro Technologies (Phoenix, AZ, USA). The capillary length is either 40 cm (30 cm to detector) or 65 cm (55 cm to detector). All measurements were performed in triplicate.

2.3. Sample handling and derivatization procedure

The derivatization procedure of amino acids with NDA in the presence of cyanide was modified from the literature [19]. It was performed in 1.5 mL centrifuge tubes. For the standard NDA-amino acid and NDA-amine derivative samples, 1.0 mL reaction mixtures (pH 9.3) containing amino acids (10 μM), amines (10 μM), NaCN (0.3 mM), NDA (0.3 mM), and sodium tetraborate (1.0 mM) were prepared. For the derivatization of amino acids and amines in MCF-7 or H184B5F5/M10 cells, 50 μL reaction mixtures (pH 9.3) containing cell lysate (24 μL), NaCN (0.8 mM), NDA (0.8 mM), and sodium tetraborate (1.0 mM) were prepared. The mixtures were allowed to react for 40 min at room temperature and, if necessary, were diluted to suitable concentrations with deionized water before injecting into the capillary.

2.4. Cell culture

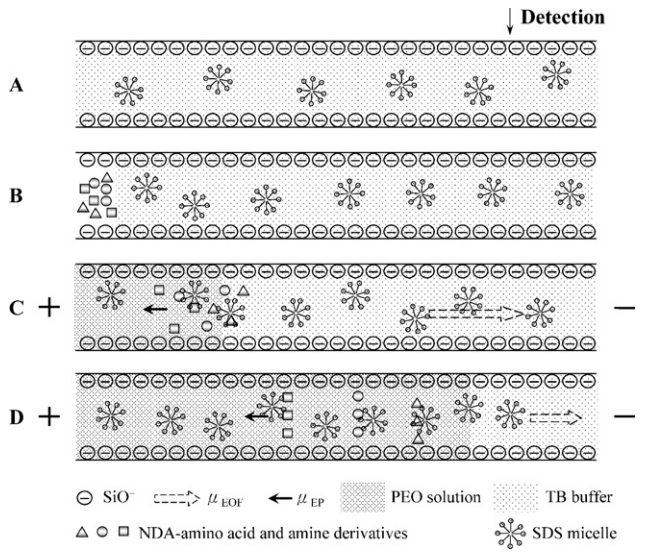
Breast cancer cells (MCF-7) and normal epithelial cell line (H184B5F5/M10) were obtained from American Type Culture Collection (Manassas, VA, USA). Both MCF-7 and H184B5F5/M10 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 100-unit penicillin/streptomycin solution. The cells were grown in bio-culture box containing CO_2 at a concentration of 5% at 37 °C. The cells were passaged each 2–3 days. A solution (Gibco) containing 0.5% (w/v) trypsin and 0.53 mM EDTA was used to lift cells from culture flasks. The cells that were placed on a hemacytometer chamber were counted using an inverted microscope with 100× magnification. Cells (2×10^6 per mL) were subjected to centrifugation at $400 \times g$ for 8 min and then washed four times with PBS (10 mL per time) to remove media. After centrifugation/wash process, aliquots of 1 mL deionized water were added to the pellets and the cells were then lysed by ultrasonication for 1 h. Centrifugal filtration of the cell lysates at $13{,}500 \times g$ using a 3K membrane was carried out to remove large molecules such as proteins. The filtrates were collected and stored at −20 °C prior to use. We calculated the concentration of amino acid per cell according to the following equation:

$$C = \frac{A}{(2 \times 10^6 \text{ cells per mL})}$$

In which, C is the mole of the amino acid/per cell and A is the concentration (mole/mL) of the amino acid determined by CE.

2.5. CE-LEDIF separation

Prior to use, capillaries were rinsed with 0.5 M sodium hydroxide for 15 min followed by with deionized water for 10 min, and



Scheme 1. Evolution of simultaneous separation of NDA-amino acid and amine derivatives by CE-LEDIF using PEO solutions in the presence of EOF. (A) The capillary was filled with 0.5 M TB buffer containing SDS. (B) NDA-amino acid and amine derivatives were hydrodynamically injected from anodic end into the capillary for 10 s. (C) When high voltage was applied, SDS micelles interacted with NDA-amino acid and amine derivatives in the sample zone. Both SDS micelles and NDA-amino acid and amine derivatives migrated against EOF and enter neutral PEO zone. (D) NDA-amino acid and amine derivatives and their aggregates with SDS micelles migrated in the PEO zone and were separated.

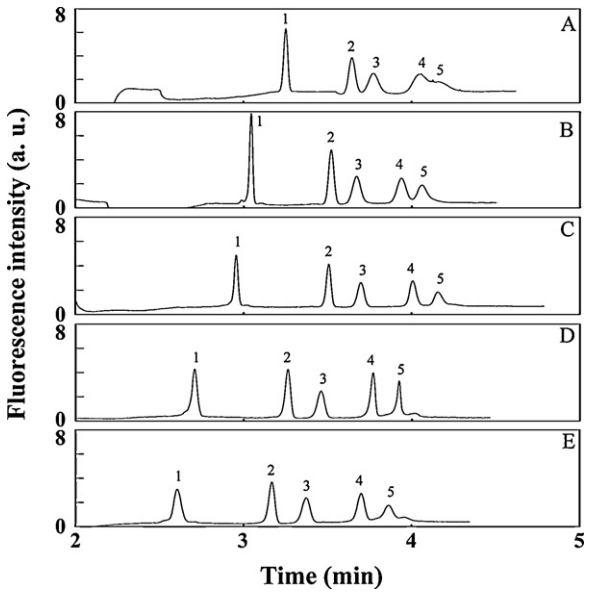


Fig. 1. Effect of SDS concentration in 0.5 M TB buffers (pH 10.2) used to fill the capillary on the separation of five analytes by CE-LEDIF at 15 kV in the presence of EOF using 0.1% PEO containing 30 mM SDS: (A) 10 mM, (B) 20 mM, (C) 30 mM, (D) 40 mM, and (E) 50 mM SDS. Capillary: 40 cm of total length, 30 cm of effective length. 0.1% PEO solution was prepared in 100 mM TB buffer (pH 9.0) containing 30 mM SDS. The mixture of analytes (each 500 nM) was diluted in deionized water. Hydrodynamic injection was conducted at 31 cm height (difference in the inlet and outlet ends) for 10 s. Peak identities: 1, Tyr; 2, Trp; 3, NE; 4, DA; 5, 5-HT.

finally with 0.5 M TB buffer (pH 10.2) containing SDS (0–50 mM) for 15 min. Hydrodynamic injection was applied at 31 cm height (the difference in the exit and entrance ends of the capillary) for 10 s (injection volume about 24 nL). After injection, the two ends of the capillary were immersed in the PEO solution and the separation was conducted at 15 kV. After each run, the capillary was equilibrated with 1.0 M TB (pH 10.2) containing SDS for 1 min, followed by TB buffer (0.5–1.5 M) containing SDS (0–50 mM) for 1 min. This treatment was quite successful to regenerate high and reproducible electroosmotic flow (EOF); RSD of the EOF was less than 0.5%. The time for the baseline shift due to detection of PEO (neutral) was used to estimate the EOF values [16].

3. Results and discussion

3.1. Effect of TB and SDS in buffers used to fill the capillary

When conducting CE separation of amino acids and amines in the presence of EOF using PEO solution, the concentrations of TB, SDS, and PEO are important [16–19]. PEO adsorption onto the surface of capillary wall through van der Waals force and hydrophilic interactions decreased upon increasing TB and SDS concentrations. We found that, relative to TB buffer, TB buffer containing SDS is superior, mainly because of faster equilibrium between runs and higher efficiency. However, use of high SDS concentrations must be prevented because high Joule heating generated causes poor reproducibility and loss in efficiency. Scheme 1 presents the separation of the NDA derivatives of amino acids and amines using PEO solutions containing SDS. In order to reduce PEO adsorption and thus to generate a high and repeatable EOF, a capillary was filled with 0.5 M TB buffer (pH 10.2) containing 10–50 mM SDS (Scheme 1A). A mixture of NDA-amino acid and amine derivatives was hydrodynamically injected from the anodic end to the capillary for 10 s (Scheme 1B). Once high voltage was applied, PEO solution entered the capillary from the anodic end. SDS micelles migrating from the zone of 0.5 M TB buffer interacted with the NDA-amino acid and amine derivatives (Scheme 1C). SDS micelles and NDA-amino acid and amine derivatives both migrated against EOF. When NDA-amino acid and amine derivatives and their complexes with SDS micelles migrated into the PEO zone, they were separated according to the mechanisms of MEKC (Scheme 1D).

We first investigated the role that the concentration of TB solution used to fill the capillary played in the separation of five standard analytes (Tyr, Trp, NE, DA, and 5-HT) when using 0.1% PEO solution (pH 9.0) containing 30 mM SDS. Of a series of TB buffers (0.1–1.5 M, pH 10.2) containing 30 mM SDS, 0.5 M TB buffer (pH 10.2) containing 30 mM SDS was optimal to fill the capillary for the separation, with respect to reproducibility and efficiency. When using low concentrations of TB buffers (<0.2 M, pH 10.2) containing 30 mM SDS, EOF was irreproducible (RSD > 3.0%) and the peaks were relatively broad. When TB concentrations were greater than 1.0 M, EOF values were relatively smaller due to the decreased zeta potential.

The role that SDS in 0.5 M TB solutions (pH 10.2) played in determining EOF and efficiency was also investigated. Fig. 1 displays that the separations of five analytes were completed within 5 min when using TB buffers containing SDS at the concentrations of 30, 40, or 50 mM. Upon increasing SDS concentration from 10 to 50 mM, EOF increased from 6.1 to 9.0×10^{-4} cm^2 V^{-1} s^{-1}. The viscosity values of TB solutions containing 10, 20, 30, 40, and 50 mM SDS were 9.7, 10.1, 10.3, 10.4, and 10.6 mPa s, respectively. The data suggest that the effect of viscosity on EOF could be negligible. In other words, EOF increases upon increasing SDS concentration were mainly due to the decrease in the absorption of PEO molecules on the capillary wall. We note that the separation efficiency increased as a result of minimized PEO adsorption on the capillary wall. PEO molecules interacted with NDA derivatives through hydrogen bonding and hydrophobic patch, causing band broadening [17]. The peak profile became sharper at SDS concentration greater than 20 mM that was above its critical micelle concentration. We estimated the CMC of SDS in 0.1% PEO by measuring the fluorescence of pyrene, which was about 4.0 mM. With respect to sensitivity, efficiency, and resolution, the optimal SDS concentration was 40 mM. Under this

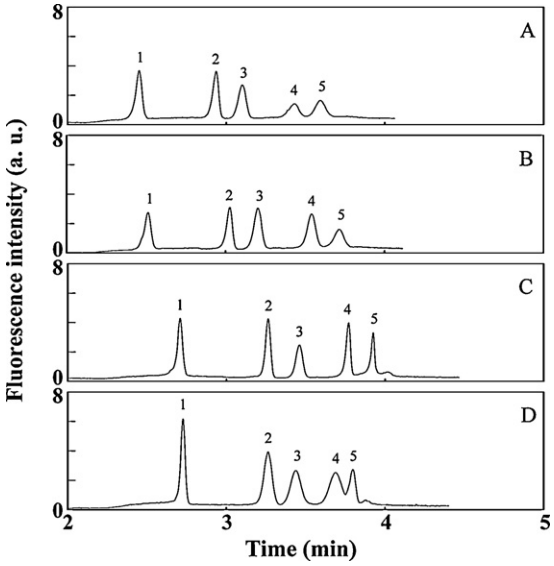


Fig. 2. Effect of PEO concentration on separation of five NDA-amino acid and NDA-amine derivatives by CE-LEDIF at 15 kV in the presence of EOF: (A) 0%, (B) 0.01%, (C) 0.1%, and (D) 0.2% PEO solutions were prepared in 100 mM TB buffer (pH 9.0) containing 30 mM SDS. Other conditions were the same as those described in Fig. 1D. Peak identities: 1, Tyr; 2, Trp; 3, NE; 4, DA; 5, 5-HT.

condition, the theoretical plates for Tyr, NE, and 5-HT (peaks 1, 3, and 5) were 6.0, 4.0, and 7.0×10^4, respectively. The RSD values ($n = 3$) of the EOF and the mobility of 5-HT were 0.43% and 0.50%, respectively.

3.2. PEO solution

In a previous study, we found that the efficiency and resolution for the NDA-amino acid derivatives improved when using PEO solutions at the concentrations larger than 0.6% [17]. Improved resolution and efficiency were mainly due to minimum analytes adsorption on the capillary wall. The NDA-amino acid derivatives (naphthalene group of the NDA) interacted with the capillary wall through hydrophobic patches, leading to irreproducibility and band broadening. In this study, we investigated the separation using PEO solutions over the concentration range 0.01–0.2% containing 30 mM SDS (Fig. 2). Use of lower concentrations of PEO solutions is beneficial for ease in preparation and operation. For comparison, a control CE-LEDIF separation result without using PEO solution is also exhibited in Fig. 2. The viscosity values of 0.1% and 0.2% PEO were 10.3 and 12.1 mPa s, respectively. Thus, the viscosity did not play a significant role in determining the electrophoretic mobility of the analytes and EOF. Although the EOF in 0.1% PEO was slightly higher than that of 0.2% PEO, the electrophoretic mobility values of the analytes in 0.1% PEO were slightly higher that of 0.2% PEO. We note that analytes migrated against EOF. Upon increasing PEO concentration (0–0.1%), the resolution for DA and 5-HT increased due to decreases in analytes adsorption on capillary wall and EOF values. When PEO concentration was 0.2%, the analyte peaks become broader, leading to loss in resolution. Thus, we chose 0.1% PEO solution for the further study.

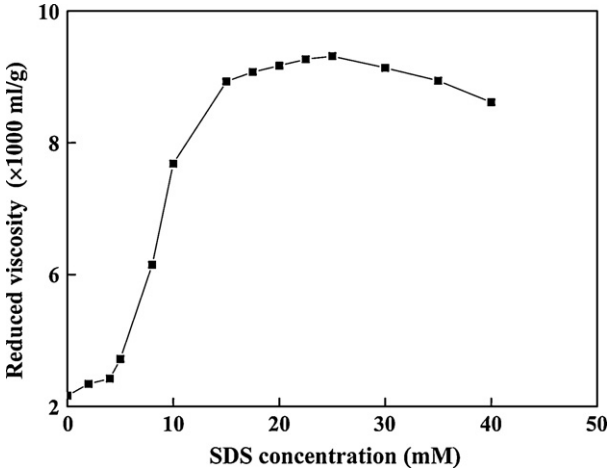


Fig. 3. A plot of reduced viscosity of 0.1% PEO as a function of SDS concentration.

We then investigated the role that SDS in 0.1% PEO solutions played in determining the separation of the five analytes when using a capillary filled with 0.5 M TB solution (pH 10.2) containing 40 mM SDS. The results are summarized in Table 1. The optimal SDS concentration in 0.1% PEO solution was 30 mM. Under this condition, the five analytes were completely separated within 5 min. To understand the role that SDS played in affecting the separation, we measured the viscosity of 0.1% PEO solutions containing various amounts of SDS (0–40 mM). The plot (Fig. 3) of the reduced viscosity of PEO solution (0.1%) as a function of SDS concentration was used to determine the critical aggregation concentration (CAC) and polymer saturated point values [25,26]. The repeatability of each point is from 5.3% to 7.2% ($n = 5$). The first significant change in the slope occurred at the SDS concentration between 4 and 5 mM; revealing that the CAC of SDS in 0.1% PEO occurred in this region. This result was consistent with the literature [27,28]. At the concentration greater than its CAC value, SDS molecules began to interact with PEO. When SDS concentrations were greater than its critical micelle concentration, SDS micelles were dominant. Further increasing SDS concentration, the number of attached micellar aggregates increased, leading to expansion of the PEO coils [29]. Upon increasing PEO concentration from 0% to 0.2%, the viscosity of PEO solutions increased. The migration times of the analytes should become longer upon increasing PEO concentration. The results (Fig. 2C and D) are not as expected when the PEO concentration was changed from 0.1% to 0.2%. The amount of free SDS micelles in 0.2% PEO solution was less than that in 0.1% PEO, leading to small electrophoretic mobility of the analytes towards the anode. We note that nonuniform electric field generated in the interface between TB and PEO solutions must be taken into account for loss in efficiency when the SDS concentration were significantly different in the two zones.

Table 1
Effect of SDS concentration in PEO solutions on peak height, theoretical plates (N), and resolution of five analytes.

SDS (mM)	Peak height (a. u.)					N ($\times 10^5$ theoretical plates)					Resolution			
	Tyr	Trp	NE	DA	5-HT	Tyr	Trp	NE	DA	5-HT	Tyr/Trp	Trp/NE	NE/DA	DA/5-HT
10	4.4	3.1	N.R.[a]	N.R	N.R.	0.14	0.07	N.R.	N.R.	N.R.	5.3	N.R.	N.R.	N.R.
20	2.9	3.1	1.8	2.3	1.6	0.11	0.18	0.13	0.20	0.31	6.2	2.5	3.1	1.8
30	5.1	5.2	2.7	3.4	1.8	0.63	0.90	0.41	0.85	0.75	19.8	4.1	5.5	2.9
40	5.2	3.6	2.6	2.6	1.7	1.06	0.67	0.44	0.76	0.91	14.1	2.7	4.9	2.7
50	4.7	2.9	2.5	2.0	1.5	0.31	0.22	0.18	0.16	0.08	8.5	1.4	2.6	1.1

[a] Not resolved.

Table 2
Amounts of identified amino acids in MCF-7 and H184B5F5/M10 cell lysates determined by CE-LEDIF.

	MCF-7					H184B5F5/M10				
	Content (fmol)	Added (fmol)	Total (fmol)	RSD (%, $n=5$)	Recovery (%)	Content (fmol)	Added (fmol)	Total (fmol)	RSD (%, $n=5$)	Recovery (%)
Ser	2.59	1.00	3.75	8.6	106	2.07	1.00	3.11	7.8	102
Gln	0.31	0.10	0.40	4.3	97	6.55	5.00	11.55	5.8	100
His	–	–	–	–	–	0.09	0.01	0.10	2.1	100
Gly	2.49	1.00	3.46	0.9	99	6.34	5.00	11.34	5.1	100
Ala	2.01	1.00	2.92	8.1	96	3.58	1.00	4.61	3.4	101
Tau	10.80	5.00	15.60	7.9	98	2.32	1.00	3.34	6.6	101
Tyr	0.74	0.10	0.80	8.4	95	2.27	1.00	3.34	7.1	103
Glu	13.97	5.00	18.69	1.3	98	22.98	10.0	33.90	8.2	104
Val	3.30	1.00	4.10	6.7	94	2.39	1.00	3.44	7.9	102
Asp	15.96	5.00	20.16	7.4	95	7.07	5.00	12.78	7.2	110

3.3. Separation of 14 NDA-amino acid and NDA-amine derivatives

In order to separate 14 NDA-amino acid and NDA-amine derivatives simultaneously, a 65-cm capillary was used. The separation was completed within 16 min as shown in Fig. 4. The RSD values of the mobilities of the analytes were all less than 0.52%. To test the quantitative practicality of this approach, a series of the NDA-amino acid and NDA-amine derivatives over the concentration range (15–1000 nM) were separately subjected to CE-LEDIF analyses. Table S1 summarizes the linearity and LOD values for the analytes. The LODs (S/N = 3) for the analytes ranged from 2.06 nM (Gly) to 19.17 nM (5-HT). Our results clearly showed the potential of the present CE-LEDIF approach for the analysis of amino acids and biogenic amines in biological samples.

3.4. Analysis of MCF-7 and H184B5F5/M10 cells

We applied the CE-LEDIF approach to the analyses of amino acids and biogenic amines in breast cancer cells (MCF-7) and normal epithelial cell line (H184B5F5/M10). Fig. 5A and B exhibit the representative electropherograms of the separations of 24 nL cell lysates of MCF-7 and H184B5F5/M10 cells, respectively. Since NDA is selective to primary amino groups, all unidentified peaks were corresponding to analytes possessing primary amino groups. By performing a standard addition method, the identified amino acids and biogenic amines in MCF-7 and H184B5F5/M10 cells were quantified. The results are summarized in Table 2. The concentrations of Gln, Tau, Glu, and NE were significantly different between H184B5F5/M10 and MCF-7 cells. The average amounts per cell of Gln in the H184B5F5/M10 and MCF-7 cells were 6.55 and 0.31 fmol, respectively. The mitochondria in malignant tumors and rapidly dividing cells have a high rate of glutamine oxidation, so the concentration of Gln in the MCF-7 cells was relatively lower than that in the normal cells. In addition, we found that the content of Tau in the MCF-7 cells was higher than that in the H184B5F5/M10 cells. Tau relates to many biochemical functions, including membrane stabilization, detoxification, antioxidation, and cell volume regulation. Cell volume is connected to cell survival, cell proliferation, and apoptosis, so Tau is an important marker for cancer cells [30,31]. Although we did not determine the concentration of NE in the cells, we detected NE in the MCF-7 cells but not in the H184B5F5/M10 cells, which agrees with a report that a high level of NE excessively stimulated its receptors on a cell, increasing the probability of immediately developing cancer within that cell [32]. Our results show great potential of the present CE-LEDIF approach for cancer study.

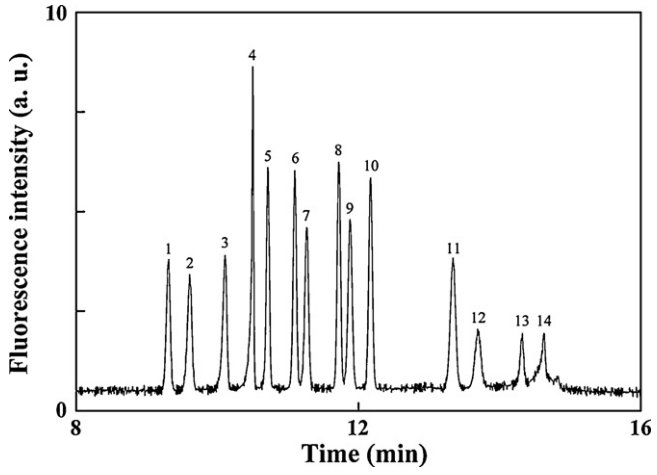


Fig. 4. Analysis of 14 standard analytes by CE-LEDIF at 15 kV in the presence of EOF using 0.1% PEO. The capillary (65 cm total length with 55 cm effective length) was filled with 0.5 M TB buffer (pH 10.2) containing 30 mM SDS. Other conditions were the same as those described in Fig. 2C. Peak identities: 1, Ser; 2, Gln; 3, His; 4, Gly; 5, Ala; 6, Tau; 7, Tyr; 8, Glu; 9, Val; 10, Asp; 11, Trp; 12, NE; 13, DA; 14, 5-HT.

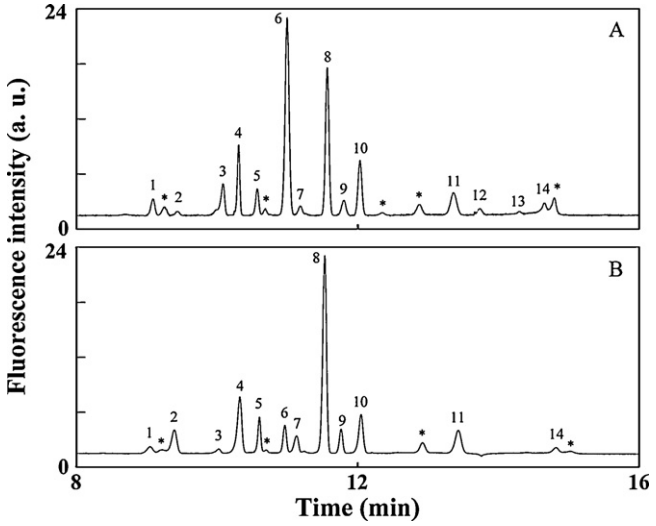


Fig. 5. Electropherograms of the lysates of (A) MCF-7 and (B) H184B5F5/M10 cells. Conditions were the same as those described in Fig. 4. Peak identities: 1, Ser; 2, Gln; 3, His; 4, Gly; 5, Ala; 6, Tau; 7, Tyr; 8, Glu; 9, Val; 10, Asp; 11, Trp; 12, NE; 13, DA; 14, 5-HT; *, unknown.

4. Conclusion

We reported a CE-LEDIF approach for the analysis of NDA-amino acid and NDA-amine derivatives simultaneously using PEO solution containing SDS. In the presence of PEO solution containing SDS, analyte adsorption on the capillary wall reduced and diffusion was lower, leading to high efficiency. This CE-LEDIF approach allowed

the separation of 11 amino acids and 3 biogenic amines within 16 min, with LODs (S/N = 3) ranging from 2.06 to 19.17 nM, and the RSD values of the mobilities less than 0.52% (n = 3). The present approach allowed quantitative detection of important amino acids and amines. The concentrations of Tau and Gln in the MCF-7 cells were higher and lower than those in the H184B5F5/M10 cells, respectively. Our present results show great potential of this CE-LEDIF approach for the study of cancer cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.11.069.

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