Talanta 88 (2012) 638–645

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

On-line concentration and separation of cationic and anionic neurochemicals by capillary electrophoresis with UV absorption detection

Ming-Mu Hsieh[a,*], En-Ping Lin[b], Shiou-Wen Huang[a]

[a] Department of Chemistry, National Kaohsiung Normal University, No. 62, Shenjhong Rd., Yanchao District, Kaohsiung City 82446, Taiwan, R.O.C.
[b] Department of Applied Chemistry and Material Science, Fooyin University, Taiwan, R.O.C

ARTICLE INFO

Article history:
Received 5 September 2011
Received in revised form
16 November 2011
Accepted 17 November 2011
Available online 25 November 2011

Keywords:
Capillary electrophoresis
Polyethylene oxide
Stacking
Neurochemicals
Glycerol

ABSTRACT

This paper presents on-line simultaneous concentration and separation of cationic and anionic neurochemicals by capillary electrophoresis (CE) with UV absorbance spectroscopy. Neurochemical stacking exploits differences in local electric field and viscosity between the sample zone and the background electrolyte (BGE). To achieve these discontinuous conditions for CE, neurochemicals were prepared in a solution containing 1 mM formic acid and 20% (v/v) acetonitrile (ACN). The capillary was filled with a solution of 500 mM Tris–borate (TB) and 10% (v/v) glycerol. The buffer vial contained 500 mM TB and 0.5% (v/v) polyethylene oxide (PEO). After injecting a large sample volume, PEO enters the capillary by electro-osmotic flow (EOF). Anionic neurochemicals stacked at the sample zone and PEO-containing BGE boundary. Simultaneously, cationic neurochemicals were concentrated at the boundary between the sample zone and the glycerol-containing BGE. The concentrated cationic neurochemicals were baseline separated in the presence of glycerol, mainly due to hydrogen bonding interactions between glycerol hydroxyl groups and the neurochemical's hydroxyl and amino groups. Under optimal stacking conditions, we observed the following: (a) the maximum sample injection volume was 720 nL; (b) the limit of detection for signal-to-noise ratio of 3 ranged from 14.7 to 313.4 nM; and (c) sensitivity enhancements compared to normal injection (15 nL) ranged from 116 to 281-fold. We evaluated the proposed method by the determination of neurochemicals in urine samples.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Neurochemicals, such as serotonin (5-HT), DL-Tryptophan (DL-Trp), and dopamine (DA) are responsible for the transmission of signals between a neuron and a target cell. The determination of neurochemicals in biological samples provides information important to pheochromocytoma, neuroblastoma, and Parkinson's disease [1]. Changes in the levels of neurochemicals are highly associated with stress, heart disease, and high blood pressure [2–4]. Thus, developing a rapid and sensitive method for the detection of neurochemicals in biological fluids is clinically important.

Currently available methods for the determination of neurochemicals include gas chromatography [5,6], high-performance liquid chromatography [7–9], and capillary electrophoresis [10]. These separation methods are often used in tandem with UV absorbance, mass spectrometry, fluorescence detection, and electrochemical detection. Of these methods, capillary electrophoresis (CE) combined with UV absorbance detection is one of the most common separation methods for the analysis of neurochemicals because it offers attractive features such as short analysis time, high resolution, small sample requirements, and no requirement for derivatization [11]. However, the determination of neurochemicals by CE-UV still suffers from low sensitivity [12–14]. For example, CE-UV commonly provides limits of detection (LODs) of 10^{-6}–10^{-7} M for catecholamines [14]. CE, in conjugation with UV laser-induced native fluorescence detection, can effectively improve detection sensitivity relative to CE-UV without the need for a derivatization step [15,16]. However, fluorescence quantum yields of neurochemicals such as catecholamines and indolamines are highly susceptible to solution pH [16]. Moreover, this system uses an expensive UV laser.

In addition to the use of CE with laser-induced native fluorescence, the combination of on-line preconcentration methods and CE provides an alternative strategy for the detection of trace neurochemicals. To date, numerous on-line concentration methods, including field-amplified sample stacking [17,18], dynamic pH junction [19,20], sweeping [21,22], and polymer-based stacking [23,24] have been developed to enhance the detection sensitivity

Abbreviations: TA, DL-tryptamine; DL-Try, DL-Tryptophan; 5-HT, 5-hydroxytryptamine; DA, dopamine; E, epinephrine; DOPA, Dopa; 5-HIAA, 5-hydroxyindole-3-acetic acid; VMA, vanillylmandelic acid; 3-IXS, 3-indoxyl sulfate; BGE, background electrolyte.

* Corresponding author. Tel.: +886 7 717 2930x7121; fax: +886 7 605 1083.
E-mail address: t3644@nknucc.nknu.edu.tw (M.-M. Hsieh).

0039-9140/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2011.11.050

of neurochemicals for CE. Each method uses differences in physicochemical properties (such as ionic strength, pH, and viscosity) between the sample zone and background electrolyte. Increasing background electrolyte concentration, diluting the sample solution, or adding organic solvent to the sample solution, or combinations of these methods, are effective approaches to induce field-amplified sample stacking [17,18]. A dynamic pH junction between the sample zone and background electrolyte is useful to focus catecholamines by changes in the local velocity [19,20]. At an extremely low electro-osmotic flow (EOF), sodium dodecyl sulfate (SDS) is capable of sweeping neurochemicals by the formation of neurochemical-SDS complexes [21,22]. Using polymer and polyelectrolyte additives in CE concentrates neurochemicals between the sample zone and polymer and polyelectrolyte because of the viscosity difference between the two zones [23,24].

This study presents a description of a novel CE method for simultaneous stacking of cationic and anionic neurochemicals, by combining polymer-based and field-amplified sample stacking of catecholamine, indolamine, and metanephrine neurochemicals. The mechanism for stacking anionic neurochemicals is based on differences in the local electric field between the sample zone and the background electrolyte (BGE), and on viscosity differences between the sample zone and BGE containing polyethylene oxide (PEO). Improvements in cationic neurochemical resolution were gained by adding glycerol to the BGE. We investigated the effects of varying glycerol and PEO concentrations on separation and stacking efficiencies, respectively. The method was evaluated by the determination of neurochemicals in urine samples.

2. Materials and methods

2.1. Chemicals and preparation

Tryptamine (TA), 5-HT, 3-methoxytyramine (3-MT), normetanephrine (NMN), 4-hydroxy-3-methoxybenzylamine (HMBA), DA, DL-Tryptophan (DL-Trp), catechol (CA), 3-(3,4-dihydroxyphenyl)-DL-alanine (DL-Dopa), 5-hydroxyindole-3-acetic acid (5-HIAA), 3-indoxyl sulfate (3-IXS), metanephrines, and vanillylmandelic acid (VMA) were obtained from Sigma–Aldrich Corporation (St. Louis, MO). Acetonitrile (ACN) and sodium hydroxide were purchased from Aldrich (Milwaukee, WI, USA). Tris(hydroxymethyl)aminomethane (Tris) was a product from J.T. Baker (Phillipsburg, NJ, USA). Boric acid was purchased from Riedel-deHaën (Buchs, Switzerland). A solution of 500 mM Tris–borate (TB) was prepared by dissolving 30.29 g of Tris and 3.19 g of boric acid in 500-mL aqueous solution. The pH of the resulting solution was 9.0. Another, 5%, 10%, 15% (v/v) glycerol and 0.1, 0.25, 0.5, 0.75, 1.0% (w/v) PEO were each of prepared in 500 mM TB solutions (pH 9.0).

2.2. Instrument

A commercial UV absorbance detector (ECOM, Germany) was performed at 220 nm for analytes, respectively. Electrophoresis was driven by a high-voltage power supply (Bertan, Hicksville, NY, USA). The high-voltage end of the separation system was put in a laboratory-made plexiglass box for safety. Data acquisition (10 Hz) and control were performed using DataApex Software (DataApex, Prague, Czech Republic). The fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were 50 cm length (10-cm to detector) with 75 μm I.D. and 365 μm O.D.

2.3. Separation and stacking

To obtain high electroosmotic flow (EOF), new fused-silica capillaries were filled with 0.5 M NaOH solution overnight. Prior to separation, the capillary was filled with a solution containing 500 mM TB (pH 9.0) and 0–15% glycerol. A mixture of neurochemicals was injected by hydrodynamic injection at 25-cm height for 10–240 s. Note that neurochemicals were prepared in a solution containing1 mM formic acid and 20% ACN solution. The ends of the capillary were immersed in the cathodic and anodic vials that both contain 500 mM TB (pH 9.0) and 0–1% PEO. When the separation was conducted at 300 V/cm, the PEO solution entered into the capillary from the anodic vial with the help of the EOF. Because the EOF was greater than the effective electrophoretic mobilities of neurochemicals, the detection window was located at 10 cm from the cathode end. Note that a neutral marker, ACN, generated a indirect peak in the electropherogram. After each run, PEO molecules adsorbed on the capillary wall were flushed out and the capillary was re-equilibrated with 0.5 M NaOH at 1 kV for 10 min. A capillary was re-filled with a solution containing 500 mM TB and 0–10% glycerol. This treatment was quite successful to regenerate a high and reproducible EOF [18]. The reproducibilities of migration time and peak area were obtained for five successive injections of standard solutions.

Under the optimal separation conditions, 15–720 nL of sample was injected hydrodynamically by raising the injection end of the capillary 10-cm above the detection end. The injection volume was determined by monitoring the baseline shift at detection window during the sample injection. The sensitivity enhancement is calculated using the following equation:

$$\text{Sensitivity enhancement} = \left(\frac{\text{LOD}_{\text{normal injection}}}{\text{LOD}_{\text{stacking}}} \right).$$

The numerator is the LOD obtained by stacking via 10% glycerol and 0.5% PEO solutions and the denominator is the LOD obtained from the normal injection method (25-cm height for 10 s.).

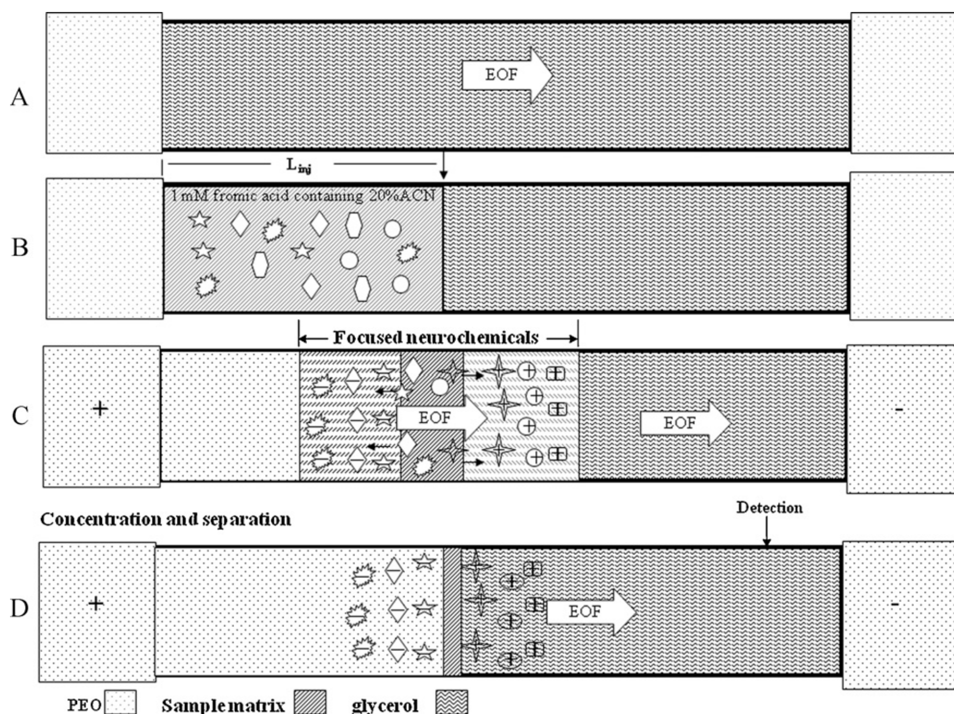
2.4. Analysis of real sample

Urine samples (500 μL) collected from a healthy adult male were diluted into two-fold with 1 mM formic acid containing 20% ACN. This treatment has been shown to be efficient for removing proteins from urine [22]. The supernatant was obtained by centrifugation of the diluted sample at 3000 rpm for 10 min. We analyzed the obtained supernatant by the proposed method.

3. Results and discussion

3.1. Effect of varying PEO, Tris–borate (TB), and glycerol concentrations

PEO-based stacking is capable of stacking various biomolecules, including DNA [23], RNA [24], protein [25], amino acid [26], catecholamines [27], and indolamines [28]; it provides approximately 100–1000-fold sensitivity improvements. The method is appropriate for the quantification of biomolecules in high-salt matrixes such as urine [18], cerebrospinal fluid [26], cancer cells [29], blood, and red blood cells [30]. However, this stacking method is limited to the analysis of anionic analytes because the velocity of neutral PEO is the same as that of EOF. In other words, cationic analytes are unable to migrate into PEO zone when PEO enters into the capillary with the help of EOF. To circumvent this problem, we filled the capillary with 500 mM TB containing 10% glycerol, while the buffer vial contained PEO solution (Scheme 1A). Upon injecting a large volume of the sample (Scheme 1B), differences in the local electric field between the sample zone and TB cause a concentration of the cationic neurochemicals while the separation process is underway (Scheme 1C). In addition to field-amplified sample stacking, anionic neurochemicals can stack through the difference



Scheme 1. Evolution of analyte zone in the separation and stacking of neurochemicals. (A) Filling of capillary with 500 mM TB (pH 9) containing different concentration glycerol; (B) injection of a large volume of anlaytes solution; (C) stacking of twelve analytes by glyerol and PEO; (D) separation of the stacking cationic and anionic neurochemicals by glycerol and PEO. The μ_{EOF} and μ_{EP} represent the EOF mobility and the electrophoretic mobilities of cationic and anionic neurochemicals, respectively.

between sample zone and PEO viscosity (Scheme 1C). The stacking cationic and anionic analytes migrate toward the detector with the EOF (Scheme 1D).

We investigated the effect of PEO concentration on stacking and separation efficiency. When the capillary and buffer vials each contained 500 mM TB, six neurochemicals dissolved in a solution containing 1 mM formic acid and 20% acetonitrile (ACN), with a viscosity equal to 0.89 cP and an injected volume of 270 nL, produced two broad peaks in the CE-electropherograms (Fig. 1A). Thus, capillary zone electrophoresis was unable to separate and stack the cationic and anionic neurochemicals under these conditions. Following the addition of 0.1% PEO to the buffer vial, three peaks appeared in the absorbance electropherograms, and a reduction occurred in the EOF (Fig. 1B). We attribute the

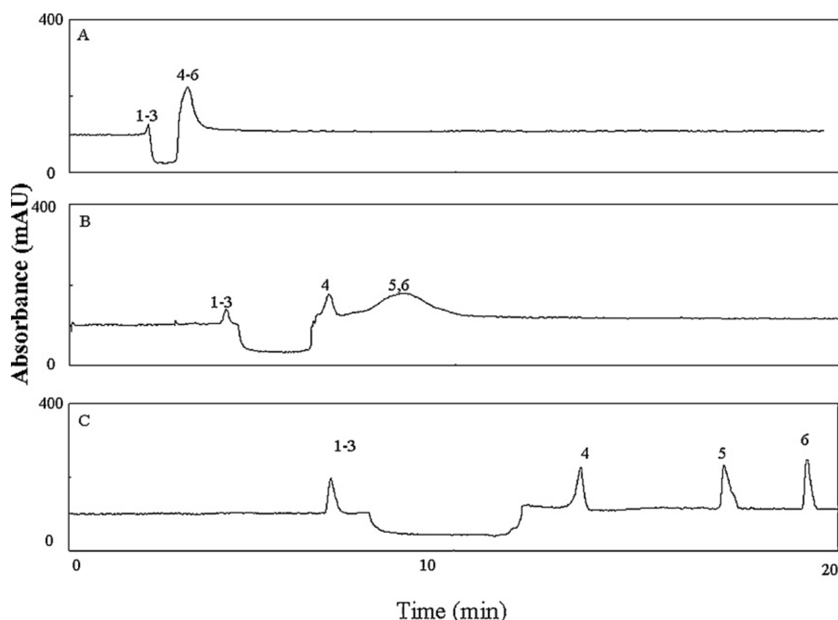


Fig. 1. Stacking and separation of cationic and anionic neurochemicals by the use of 500 mM TB (pH 9) containing (A) 0%, (C) 0.1%, and (D) 0.5% (w/v) PEO solutions as additives. Before separation, the capillary was filled with 500 mM TB (pH 9) solution, while buffer vials contains 0–0.5% (w/v) PEO, electrophoresis conditions: 50-cm capillary (10-cm to detector); applied voltage, 15 kV; hydrodynamic injection at 25-cm height for 10 s and direct UV detection at 220 nm. Peak identities: 1, 5-HT (50 μM); 2, HMBA (100 μM); 3, DA (100 μM); 4, DL-Trp (15 μM); 5, DL-Dopa (100 μM); 6, 5-HIAA (25 μM).

Table 1
Effects of PEO on EOF, resolution and peak height of three model analytes.

PEO (%)[a]	EOF ($\times 10^{-4}$ cm^2 v^{-1} S^{-1}) (RSD%; $n = 5$)	Resolution		Peak height (mAU)		
		Trp/L-Dopa	L-Dopa/5-HIAA	Trp	L-Dopa	5-HIAA
0	2.95 (1.8)	3.07	1.58	51,000	84,000	54,000
0.1	2.67 (1.9)	4.52	2.41	72,000	87,000	59,000
0.25	2.39 (2.2)	9.17	4.34	106,000	96,000	61,000
0.5	2.04 (2.3)	9.62	5.15	109,000	130,000	190,000
0.75	1.99 (2.7)	10.99	6.39	88,000	110,000	170,000
1.0	1.80 (3.1)	12.62	7.59	78,000	100,000	160,000

[a] The capillary was filled with 500 mM TB (pH 9) containing 10% glycerol solution, while buffer vials contains 0–1.0% (w/v) PEO. Other conditions are the same as in Fig. 1.

reduced EOF to PEO adsorption on the capillary surface [31]. As shown in Fig. 1C, increasing PEO concentration to 0.5% successfully separated and concentrated three anionic neurochemicals Trp ($pK_a = 2.46$, 9.41), Dopa ($pK_a = 8.72$), and 5-HIAA ($pK_a = 4.7$). The improvement in stacking efficiency was due to the increase in BGE viscosity. The improvement in separation efficiency could be explained by the decrease in EOF [31], and by hydrogen-bonding interactions between PEO polyethylene glycol chains and neurochemical hydroxyl and carboxylic groups [18]. A previous study demonstrated the separation of a mixture of Trp and 5-HIAA in the presence of 2% PEO [32]. Similarly, Kuo et al. reported that five neurochemicals (TA, 5-HT, Trp, Dopa, and epinephrine) were baseline separated by exploiting hydrogen-bonding interactions between hydroxyl groups on the surfaces of SiO_2 nanoparticles and neurochemical hydroxyl and carboxylic groups [33]. However, under identical conditions, three cationic neurochemicals 5-HT ($pK_a = 9.8$), HMBA ($pK_a = 10.1$), and DA ($pK_a = 10.6$) were concentrated, though not resolved. The three cationic compounds did not migrate into the PEO zone, possibly because filling the capillary with PEO-containing BGE prior to separation causes significant decay of the EOF. Table 1 shows that 0.5% (v/v) PEO in BGE is optimal for separating and stacking Trp, Dopa, and 5-HIAA.

Filling the capillary with PEO causes the EOF to decay with time. Thus, to separate a solution of 5-HT, HMBA, and DA cationic neurochemicals, hydroxyl-containing additives were used to supplant PEO, and the capillary was filled with a high concentration (10%) of hydroxyl-containing additives. By contrast with using methanol and ethanedial as additives, filling the capillary with a solution of 500 mM TB and 10% glycerol resolved three discreet peaks representing 5-HT, HMBA, and DA (Fig. 2). This suggests that the separation efficiency of cationic neurochemicals is improved by increasing the number of hydroxyl groups. As well as using PEO, cationic neurochemicals are separable by using hydrogen-bonding interactions between hydroxyl groups of the glycerol hydroxyl, and amino groups of neurochemicals. We tested the effect of glycerol concentration on the separation of three cationic and three anionic neurochemicals. A comparison of Fig. 3 with Fig. 1C shows that using greater than 5% glycerol concentration provides baseline separation of the cationic compounds 5-HT, HMBA, and DA. Because the addition of different concentrations (0–15%) of glycerol to a solution of 500 mM TB caused the change in viscosity from 1.1 to 1.7 cP, we ruled out that the baseline separation of the three cationic neurochemicals using glycerol is due to the change in solution viscosity. Moreover, the mobility of DA was found to be close to EOF, indicating the formation of the complexes of endiol-containing DA and borate [34]. In other words, a decrease in the mobility of DA is due to that borate can from five or six-membered cyclic esters with DA. However, the separation efficiency of the three anionic neurochemicals was insensitive to the concentration of glycerol. Fig. 4 shows that the EOF increased with increasing the glycerol concentration, demonstrating that glycerol is capable of reducing the extent of PEO adsorption on the capillary surface. Thus, the time required for separation of the six neurochemicals was shortened by increasing the concentration of glycerol. The presence of glycerol both reduces PEO adsorption, and improves the separation efficiency for cationic neurochemicals. We employed 10% glycerol in subsequent studies to provide optimal resolution and rate.

3.2. Stacking and separation of cationic and anionic neurochemicals

To confirm the proposed stacking mechanism, we varied the sample volume in the range 15–720 nL under optimal separation conditions for rate and resolution. The total capillary volume was 2650 nL (60 cm length, 75-μm I.D.). Electropherogram-a (Fig. 5A) was obtained using normal injection conditions of 25-cm height

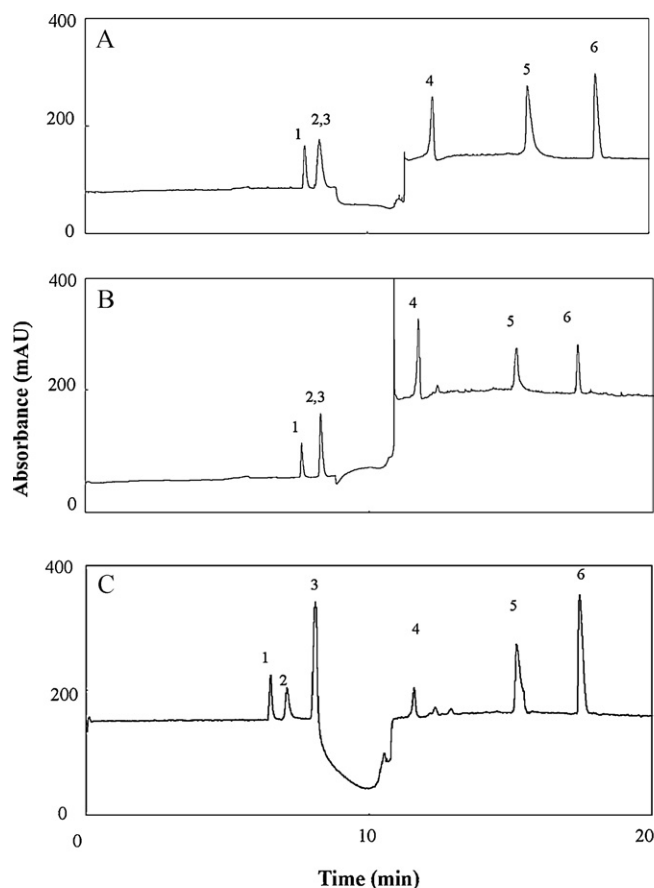


Fig. 2. Capillary was filled with 500 mM TB containing (A) 10% (v/v) methanol (B) 10% ethanediol (C) 10% glycerol solution (pH 9.0), while buffer vials contains 0.5% (w/v) PEO, The other conditions are the same as Fig. 1.

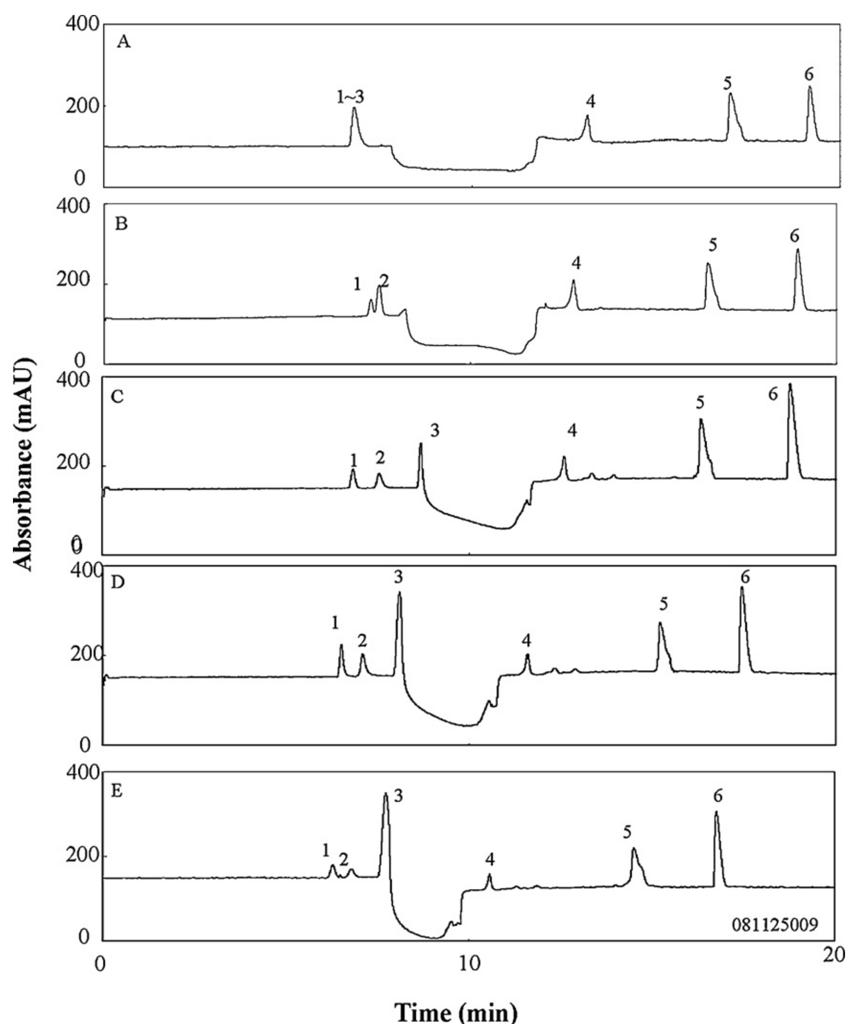


Fig. 3. Capillary was filled with 500 mM TB containing (A) 0% (B) 0.1%(C) 5% (D) 10% (E) 15% (v/v) glycerol solution (pH 9.0), while buffer vials contains 0.5% (w/v) PEO, The other conditions are the same as Fig. 1.

for 5 s to provide a 15 nL injection volume. The figure shows twelve tiny peaks corresponding to TA, 5-HT, 3-MT, HMBA, NMN, DA, Trp Dopa, catechol, 5-HIAA, VMA, and 3-IXS. The LODs at a signal to noise (*S*/*N*) ratio of 3 for these peaks are estimated to be 3.6, 15.6, 27.7, 31.3, 28.5, 40.1, 3.8, 25.6, 33.3, 7.3, 41.7, and 2.8 μM, respectively. Electropherograms b–e in Fig. 5A reveal that the twelve peak areas significantly increased with increasing sample injection time, and reached a plateau at 240 s (720 nL; 27% of the capillary volume) without loss of resolution. We found good linearity between peak height and injection time (5–240 s) (Table 2), indicating that the proposed method allowed good stacking of cationic and anionic neurochemicals. For injection times exceeding 240 s, the peak efficiency of two neurochemicals declined (electropherogram e in Fig. 5A). The injection volume of this stacking method was confined to 720 nL, and electropherograms a–e in Fig. 5A show that the separation time increases with an increase in sample volume. PEO

Table 2
On-line concentration and separation of biogenic amines and their metabolites using discontinuous viscosities solutions.

Amines	Linear regression[a]	Linear regression coefficient (R^2)	LOD (nM) ($S/N = 3$)[b]	Sensitivity enhancement
TA	$y = 218.98x + 1479.8$	0.9946	31.4	116
5-HT	$y = 385.32x + 3755.7$	0.9963	83.3	187
3-MT	$y = 537.28x + 1056.6$	0.9937	98.7	281
HMBA	$y = 444.77x + 1642.9$	0.9978	131.6	238
NMN	$y = 415.66x + 1007.9$	0.9991	154.4	185
DA	$y = 1044.9x + 16496$	0.9915	204.2	197
Trp	$y = 538.6x + 5561.6$	0.9938	17.3	216
DL-dopa	$y = 560.02x + 8758.1$	0.9949	184.2	140
Catechol	$y = 611.16x + 7278.7$	0.9924	163.5	204
5-HIAA	$y = 677.98x + 10391$	0.9905	30.3	241
VMA	$y = 536.24x + 10749$	0.9918	313.4	133
3-IXS	$y = 482.31x + 5517.2$	0.9913	14.7	190

[a] *y* is peak area; *x* is the e injection time (s) at 20 cm-height for 5–240 s, capillary length 60 (50) cm.
[b] Estimated from Fig. 4B.

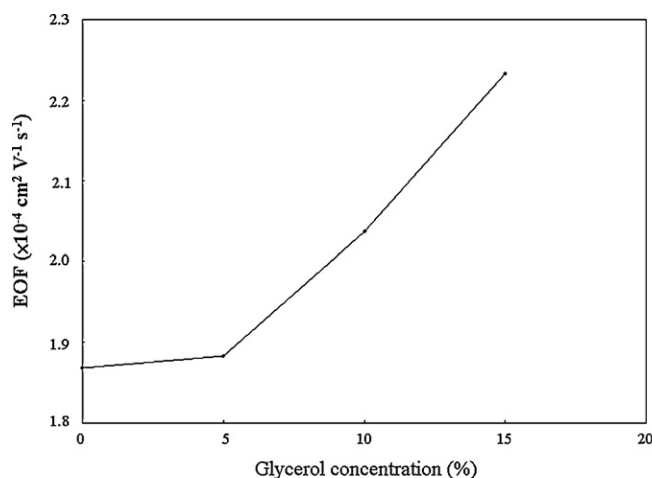


Fig. 4. Effect of the concentration of glycerol buffers on the EOF in 0.5% PEO at 15 kV. PEO solutions were prepared in 500 mM TB buffers, pH 9.0. Capillary, 50 cm total length, 40 cm effective length, filled with 500 mM TB, pH 9 containing a variety glycerol buffers.

adsorption to the capillary is relatively strong in low-conductivity solutions [35]; thus, injection of a large sample volume (1 mM formic acid and 20% ACN) resulted in significant PEO adsorption. We therefore attribute the observed increase in separation time to the decay in EOF. Injection of a large sample-induced EOF decay was observed in the case of the use of PEO for the stacking and separation of DNA [35], proteins [30], and amino acids [36].

Under optimal stacking conditions (240 s, 720 nL), LOD values of the twelve neurochemicals (*S*/*N* of 3) ranged from 14.7 to 313.4 nM (Table 2; estimated from Fig. 5B). These values correspond to sensitivity enhancements, ranging from 116 to 281-fold compared to sensitivities obtained using normal injection methods (Table 2). Although this method only provides moderate sensitivity enhancements, the retained resolution and separation efficiency are more important than focusing on the consideration on sensitivity enhancement alone.

3.3. Analysis of neurochemicals in urine

The high resolution and sensitivity obtained by using the proposed method was applied to the determination of neurochemicals in urine. Fig. 6A shows the analysis of 10-fold diluted urine samples under normal injection conditions (25 nL), while Fig. 6B displays the analysis results of identical samples under stacking conditions (600 nL). A comparison of Fig. 6B (stacking) and Fig. 6A (non-stacking) reveals several new peaks (marked by arrow bars) resulting from the stacking of urine samples. The result indicates that the proposed method successfully detects relatively low neurochemical concentrations present in a complex matrix. By comparing peak area both with (Fig. 6C) and without (Fig. 6B) spiked standard neurochemicals, we identified these peaks (indicated by number) as corresponding to TA, 5-HT, 3-MT, HMBA, Trp, DA, 5-HIAA, VMA, and 3-IXS. The standard addition method was conducted to quantitatively determine the concentrations of the seven separated compounds (TA, 5-HT, 3-MT, Trp, DA, 5-HIAA, and VMA) in urine. As shown in Table 3, a plot of peak area against spiked concentration exhibits good linearity ($R^2 > 0.9910$). The migration time intraday and interday precisions for the seven neurochemicals fell in the ranges 1.6 to 4.0% and 2.0 to 6.6%, respectively. The mean recoveries for the eight neurochemicals at three spiked levels (1, 25, and 75 μM) were from 93 to 107%. In separate experiments, the concentrations of TA, 5-HT, 3-MT, Trp, 5-HIAA, VMA, and 3-IXS in urine samples were determined to be 0.36 ± 0.1, 0.12 ± 0.1, 0.30 ± 0.24, 59.5 ± 1.01, 41.5 ± 2.21, 11.4 ± 1.55, and 20.4 ± 2.68 μM, respectively. Previous studies reported that the normal concentrations of TA, 5-HT, 3-MT, Trp, VMA, 5-HIAA, and

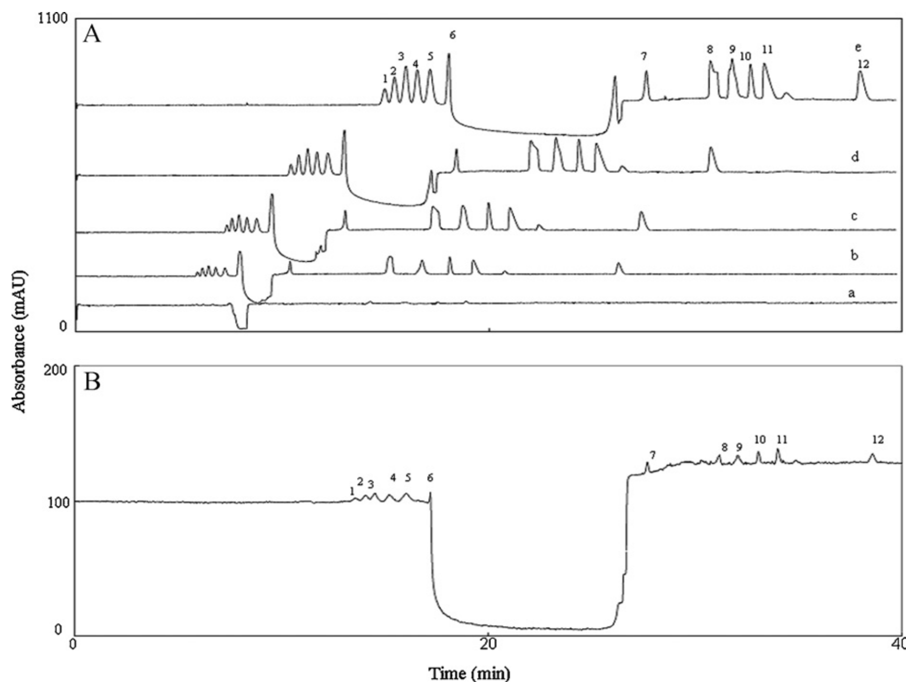


Fig. 5. (A) Stacking and separation of (a) 15, (b) 180, (c) 360, (d) 540 and (e) 720 nL twelve analytes by discontinuous buffer in the presence of 10% glycerol, 0.5% PEO. Peak identities: 1, TA (10 μM); 2, 5-HT (50 μM); 3, 3-MT (100 μM); 4, HMBA (100 μM); 5, NMN (100 μM); 6, DA (100 μM); 7, DL-Trp (15 μM); 8, DL-Dopa (100 μM); 9, CA (100 μM); 10, 5-HIAA (25 μM); 11, VMA (100 μM); 12, 3-IXS (10 μM). (B) Stacking and separation of 720 nL twelve analytes by discontinuous buffer in the presence of 10% glycerol, 0.5% PEO. Peak identities: 1, TA (0.25 μM); 2, 5-HT (0.5 μM); 3, 3-MT (0.625 μM); 4, HMBA (2.5 μM); 5, NMN (2.5 μM); 6, DA (5 μM); 7, DL-Trp (0.375 μM); 8, DL-Dopa (2.5 μM); 9, CA (2.5 μM); 10, 5-HIAA (0.625 μM); 11, VMA (2.5 μM); 12, 3-IXS (0.25 μM). Electrophoresis conditions: 60-cm capillary (10-cm to detector), applied voltage, 18 kV. The other conditions are the same as Fig. 1.

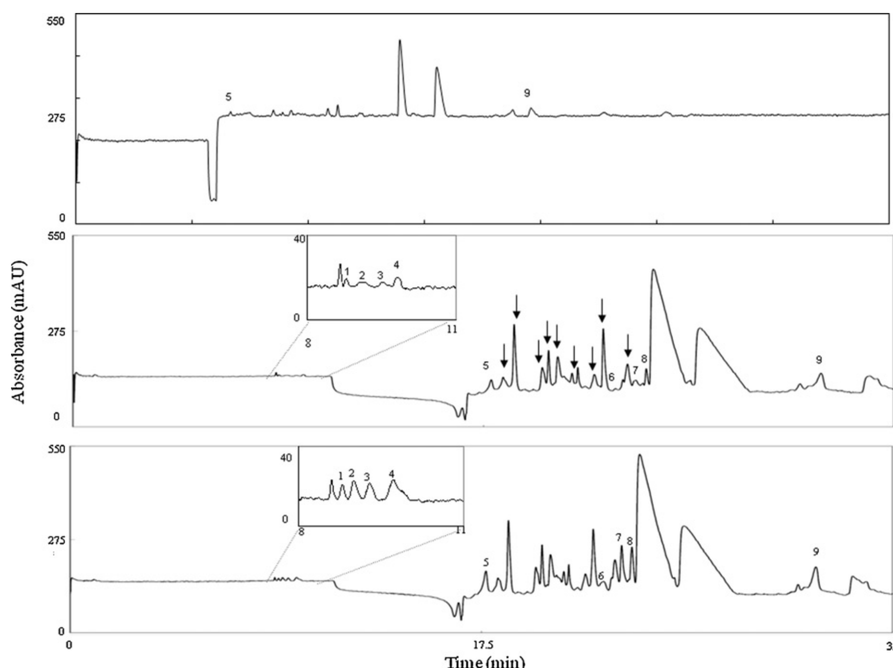


Fig. 6. On-line concentration and separation of urine sample by CE with UV detection. Urine samples were diluted by a factor of 10 using 1 mM formic acid containing 20% acetonitrile and then injected by raising the capillary inlet to 25 cm height for (a) 5 s and (b) 180 s. The new peaks were indicated by arrow bars. (c) Urine samples were spiked with TA (0.5 μM), 5-HT (2.5 μM); 3-MT (5 μM), HMBA (5 μM); DL-Trp (5 μM); CA (25 μM), 5-HIAA (10 μM), VMA (20 μM), 3-IXS (2.5 μM) and then injected by raising the capillary inlet to 25 cm height for 180 s. Peak identifies: 1, TA; 2, 5-HT; 3, 3-MT; 4, HMBA; 5, Trp; 6, CA; 7, 5-HIAA; 8, VMA; 9, 3-IXS. The other conditions are the same as those in Fig. 5.

Table 3
The quantifications of 7 biogenic amines and their metabolites by CE-UV when the sample volume was injected up to 600 nL.

Compounds	Spiked concentration (μM)	Linear regression	Linear regression coefficient (R^2)	Unknown concentration (μM)[d]	Mean recovery (%)[a]	Variation (%)	
						Intraday[b]	Interday[c]
TA	1–10	$y = 1854.6x + 672.2$	0.9958	0.36	106	4.1	2.8
5-HT	1–10	$y = 5397.6x + 644.9$	0.9963	0.12	109	4.4	3.1
3MT	1–10	$y = 2528.1x + 753.8$	0.9984	0.30	93	6.6	4.0
Trp	10–75	$y = 8639.6x + 51422$	0.9988	59.5	101	2.8	2.1
5-HIAA	10–75	$y = 3977.3x + 16525$	0.9957	41.50	94	4.7	3.3
VMA	10–75	$y = 19986x + 22743$	0.9988	11.40	99	3.1	2.2
3-IXS	1–10	$y = 4700.5x + 95867$	0.9991	20.40	102	2.0	1.6

[a] The recoveries were determined in triplicate at the spiked concentrations of 1, 25 and 75 μM. in urine sample.
[b] The samples were analyzed six consecutive times in 1 day (intraday, $n = 5$).
[c] The samples were analyzed five consecutive times on five different days (interday, $n = 25$).
[d] Refs. [33,37,38].

3-IXS in urine are less than 0.4, 0.9, 1.2, 604, 45, 50, 5, and 660 μM, respectively [3,33,37,38]. Thus, our results conform to the reported values.

4. Conclusions

We have demonstrated a method for the simultaneous on-line concentration and separation of cationic and anionic neurochemicals, based on differences in the local electric field between the sample zone and TB-containing BGE, and viscosity differences between the sample zone and PEO-containing BGE. When the capillary was filled with 10% glycerol prior to separation, the separation efficiency and rate were enhanced significantly. Under optimal stacking conditions, sensitivity improvements of 116- to 281-fold were obtained, providing LODs at nM concentrations for the analysis of twelve cationic and anionic neurochemicals. The pretreatment of urine samples with organic solvents is important for the success of analyzing compounds such as TA, 5-HT, 3-MT, Trp, 5-HIAA, VMA, and 3-IXS by the stacking method. Our findings suggest that this method holds great potential for diagnostic applications and neuron studies.

Acknowledgment

This work was supported by the National Science Council of Taiwan under contracts NSC 99-2113-M-017-004-MY2.

References

[1] K. Vuorensola, H. Sirén, J. Chromatogr. A 895 (2000) 317–327.
[2] J. Bergquist, A. Ściubisz, A. Kaczor, J. Silberring, J. Neurosci. Methods 113 (2002) 1–13.
[3] D.M. Paquette, R. Sing, P.R. Banks, K.C. Waldron, J. Chromatogr. B 714 (1998) 47–57.
[4] M. Du, V. Flanigan, Y. Ma, Electrophoresis 25 (2004) 1496–1502.
[5] M.Y. Khuhawar, A.A. Memon, P.D. Jaipal, M.I. Bhanger, J. Chromatogr. B: Biomed. Sci. Appl. 723 (1999) 17–24.
[6] B. Dorhout, A.W. Kingma, E. de Hoog, F.A.J. Muskiet, J. Chromatogr. B: Biomed. Sci. Appl. 700 (1997) 23–30.
[7] J. Lange, K. Thomas, C. Wittmann, J. Chromatogr. B 779 (2002) 229–239.

[8] A. Ormazabal, A. García-Cazorla, Y. Fernández, E. Fernández-Álvarez, J. Campistol, R. Artuch, J. Neurosci. Methods 142 (2005) 153–158.
[9] N. Innocente, M. Biasutti, M. Padovese, S. Moret, Food Chem. 101 (2007) 1285–1289.
[10] D.A. Román, A.S. Carretero, C.C. Blanco, A.F. Gutiérrez, Biomed. Chromatogr. 18 (2004) 422–426.
[11] Y.-M. Liu, J.-K. Cheng, J. Chromatogr. A 1003 (2003) 211–216.
[12] H. Sirén, M. Mielonen, M. Herlevi, J. Chromatogr. A 1032 (2004) 289–297.
[13] M.K. Shirao, S. Suzuki, J. Kobayashi, H. Nakazawa, E. Mochizuki, J. Chromatogr. B 693 (1997) 463–467.
[14] G. Liu, J. Chen, Y. Ma, J. Chromatogr. B 805 (2004) 281–288.
[15] H.-T. Chang, E.S. Yeung, Anal. Chem. 67 (1995) 1079–1083.
[16] Y.H. Park, X. Zhang, S.S. Rubakhin, J.V. Sweedler, Anal. Chem. 71 (1999) 4997–5002.
[17] Q. Weng, G. Xu, K. Yuan, P. Tang, J. Chromatogr. B 835 (2006) 55–61.
[18] C.-C. Wang, S.-S. Chiou, S.-M. Wu, Electrophoresis 26 (2005) 2637–2642.
[19] P. Britz-McKibbin, D.D.Y. Chen, Anal. Chem. 72 (2000) 1242–1252.
[20] J.-B. Kim, P. Britz-McKibbin, T. Hirokawa, S. Terabe, Anal. Chem. 75 (2003) 3986–3993.
[21] C.-M. Shih, C.-H. Lin, Electrophoresis 26 (2005) 962–969.
[22] H.-M. Tseng, D.A. Barrett, J. Chromatogr. A 1216 (2009) 3387–3391.
[23] M.-M. Hsieh, W.-L. Tseng, H.-T. Chang, Electrophoresis 21 (2000) 2904–2910.
[24] P.L. Chang, Y.S. Chang, J.H. Chen, S.J. Chen, H.-C. Chen, Anal. Chem. 80 (2008) 8554–8560.
[25] W.L. Tseng, H.T. Chang, Anal. Chem. 72 (2000) 4805–4811.
[26] P.L. Chang, T.C. Chiu, H.T. Chang, Electrophoresis 27 (2006) 1922–1931.
[27] C. Cakal, J.P. Ferrance, J.P. Landers, P. Caglar, Anal. Bioanal. Chem. 398 (2010) 1909–1917.
[28] W.-L. Tseng, S.-M. Chen, C.-Y. Hsu, M.-M. Hsieh, Anal. Chim. Acta 613 (2008) 108–115.
[29] Y.-Y. Kao, K.-T. Liub, M.-F. Huang, T.-C. Chiu, H.-T. Chang, J. Chromatogr. A 1217 (2010) 582–587.
[30] C.-J. Yu, C.-L. Su, W.-L. Tseng, Anal. Chem. 78 (2006) 8004–8010.
[31] T.-C. Chiu, H.-T. Chang, J. Chromatogr. A 1146 (2007) 118–124.
[32] M.M. Hsieh, C.E. Hsu, W.L. Tseng, H.T. Chang, Electrophoresis 23 (2002) 1633–1641.
[33] I.-T. Kuo, Y.-F. Huang, H.-T. Chang, Electrophoresis 26 (2005) 2643–2651.
[34] T.L. Paxon, P.R. Powell, H.-G. Lee, K.-A. Han, A.G. Ewing, Anal. Chem. 77 (2005) 5349–5355.
[35] W.-L. Tseng, H.-T. Chang, J. Chromatogr. A 924 (2001) 93–101.
[36] K.-C. Li, M.-M. Hsieh, C.-W. Chang, E.-P. Lin, T.-H. Wu, Talanta 82 (2010) 1912–1918.
[37] H. Sirén, U. Karjalainen, J. Chromatogr. A 853 (1999) 527–533.
[38] G.T. Bryan, Am. J. Clin. Nutr. 19 (1966) 105–112.