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Chiral analysis of neurotransmitters using cyclodextrin-modified capillary electrophoresis equipped with microfabricated interdigitated electrodes

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Abstract

We present cyclodextrin-modified capillary electrophoresis equipped with a microfabricated chip consisting of an array of eight interdigitated microband platinum electrodes (IDs) for simultaneous analysis of three chiral models: epinephrine, norepinephrine and isoproterenol. The IDE chip, positioned very close to the capillary outlet, served as an amplification/detection system. Emerging neurotransmitters at the IDE surface were oxidized at +1.1 V by seven electrodes of the array and then detected by the remaining electrode, poised at +0.0 V. There was an amplification effect on the detecting electrode owing to the recycle between the reduced and oxidized forms of the optical isomers at the electrode surface. The detecting ''amplification'' current response was governed by the applied potential, the detecting electrode position, the number of adjacent electrodes used for recycling and the distance between the oxidative and reductive electrodes. The six chiral forms of the three neurotransmitters were resolved using 25 m*M* heptakis(2,6,di-*o*-methyl)-β-cyclodextrin with a detection limit of ~5 μ*M*. The scheme detected a reduced compound at a reducing potential instead of conventional oxidation detection to alleviate electrode fouling and electroactive interferences. The concurrent oxidation/reduction detection of compounds also facilitated and ascertained peak identification as interfering compounds were unlikely to have the same oxidative/reductive characteristics and mobilities as the analytes of interrogation.

Keywords: Electrodes; Chip technology; Instrumentation; Detection, electrophoresis; Enantiomer separation; Electrochemical detection; Cyclodextrins; Epinephrine; Norepinephrine; Isoproterenol; Neurotransmitters

1. Introduction

Living organisms are composed of chiral biomolecules such as amino acids, sugars, proteins and nucleic acids and show different biological responses to one of a pair of enantiomers [1]. Therefore, chiral drug enantiomers, pesticides, and waste compounds often have widely different biological/toxicological properties and efficacies [2,3]. The importance of chirality has been well recognized in the pharmaceutical/agrochemical industries, pharmacokinetic studies and organic synthetic process development [4]. Presently, the majority of commercially available drugs are both synthetic and chiral but a large number of chiral drugs are still marketed as racemic mixtures [5]. The development of enantiomers and

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racemates has placed increasing demands on analytical procedures. Capillary electrophoresis (CE), an efficient analytical technique with respect to speed and automation, has emerged as an attractive tool to assess the purity of enantiomers. In CE technology, a chiral selector is simply added to the running buffer to create or modify a chiral environment. Cyclodextrins (CDs) and their derivatives have widely been used as chiral selectors in CE [6,7]. The hydroxyl units on the top of the cyclodextrin cavity are chirally positioned and exhibit different interactions with solute enantiomers when they are included into the cyclodextrin hydrophobic cavities. The hydroxyl groups can be derivatized to give a wide range of neutral and ionic CD derivatives, which become more water-soluble and effect different chiral separations compared to their native counterparts. Consequently, the development of CE-CD for separating the optical isomers has been developed extensively. The topic is too large to elaborate here and the interested reader should refer to the literature [8–13].

To date, UV–Vis absorption is still the most frequently used detection method because of its universality. Therefore, the process of detection in CE still presents a major challenge due to the small dimensions of the capillary (25–75 μm) in comparison with the 1-cm cell in HPLC. For most important applications, on-line UV detection is at or near its limits for sensitivity and improved detection without resorting to lasers is essential [14]. Inexpensive electrochemical detection with high sensitivity and simplicity has been coupled with CE for analysis of electroactive analytes [15–21]. The dual-electrode detection scheme was used in CE for analysis of cysteine/cystine [22], catecholamines [23] and analytes that reacted with electrogenerated bromine [24]. In such a study, catecholamines could be detected while undergoing chemically reversible oxidations using a ring-disk microelectrode. The analyte was first oxidized at the disk followed by reduction at the ring electrode with a collection efficiency (the ratio of the reduction over oxidation current) of ~35%. The dual-electrode detector has also been used in conjunction with electrophoresis chips for the detection of catechol with a collection efficiency up to 44% [25]. Since the pioneering work of Wallingford and Ewing [26], these end-column configurations with or without decoupling devices containing an electrode located very close to the capillary outlet have become popular for numerous applications [27]. End-column set-ups are particularly useful for situations that require a frequent replacement of the detecting electrode or when electrode fouling can only be overcome by mechanical cleaning. However, these arrangements frequently require time-consuming ‘‘capillary-detector’’ alignment during the course of measurement to ensure reproducibility.

In principle, an electrode array detector can be easily positioned and manipulated across the channel exit to minimize frequent alignments of the detector. Each addressable electrode can also be poised at the potential most sensitive towards the target analyte. Recently, microfabricated electrode arrays have been used as electrochemical detectors with CE for the detection of dopamine [28]. Interdigitated gold microband electrodes have been used as detectors with CE [29] for the analysis of catecholamines [30]. In such a study, dopamine was measured at a single electrode, poised at +0.6 V to provide a detection limit of 13 μ*M*. Using the same interdigitated gold microbands in a dual electrode configuration for redox cycling of ferrocene derivatives, collection efficiencies of ~25% were reported with a 75-μm I.D. capillary, positioned horizontally at 75 μm from the interdigitated electrode (IDE) surface [31]. In this arrangement, there was no amplification at the oxidation electrode compared to the single electrode configuration. However, redox cycling (1.5× amplification) was observed when the capillary was 300 μm from the IDE surface. Carbon film based interdigitated ring-shaped arrays (IDRAs) have also been used as electrochemical detectors to measure catecholamines with CE [32,33]. In these methods, a redox cycle was employed between two working electrodes and detection limits were in the n*M* range. Depending upon the design, collection efficiencies for the catecholamines ranged from 18 to 65% [32] and 17–84% [33], with epinephrine being the lowest, while the number of redox cycles reported were 1.7 [32] and 3.7 [33].

This study describes cyclodextrin-modified CE coupled with IDEs for chiral analysis of two α-adrenergic agonists; epinephrine, norepinephrine and one β-adrenergic agonist; isoproterenol. The collection efficiency of the IDEs was examined for these

analytes to develop an amplification scheme for improvement of the detection sensitivity. The amplification or recycling scheme was effected by using simultaneously a combination of reduction and oxidation potentials at the IDE surface. The detection scheme monitored reduced neurotransmitters at a reduction potential instead of the conventional oxidation potential to circumvent fouling and electroactive interferences. The study also focused on the performance of the IDEs with respect to the amplification factor, reproducibility and ease of alignment.

2. Experimental

2.1. Materials

Isoproterenol hydrochloride, (*R*)-(−)-isoproterenol hydrochloride, (±)-epinephrine, (*R*)-(−)-epinephrine, (±)-norepinephrine hydrochloride and 4-morpholineethanesulfonic acid (MES) were purchased from Aldrich (Milwaukee, WI, USA). Heptakis(2,6,di-*O*-methyl)-β-cyclodextrin (DM-β-CD) and (*R*)-(−)-norepinephrine (arterenol) hydrochloride bitartrate salt were obtained from Sigma (St. Louis, MO, USA). Tris was purchased from Gibco BRL (Grand Island, NY, USA) while 85% *O*-phosphoric acid was obtained from Fisher (Fairlawn, NJ, USA).

2.2. Capillary electrophoresis (CE)

Polyimide-coated fused-silica capillaries (20-μm internal diameter, Polymicro, Phoenix, AZ) with an 80-cm capillary length (volume 250 nl) were used in this study. At ambient temperature, electrophoresis was performed at +25 kV with sample injection electrokinetically at +25 kV for 20 s (EH Glassman High Voltage, Whitehouse Station, NJ, USA). The separation buffer consisted of 50 m*M* Tris-phosphate, pH 2.5 and various concentrations of DM-β-CD. All solutions were filtered through 0.45-μm Millex-HV filters (Millipore, Bedford, MA, USA) before use. The capillary outlet was sealed with epoxy into a pipette tip so that only 6 cm protruded. The pipette tip was firmly attached vertically into a micromanipulator (model HS6, World Precision Instruments, Sarasota, FL, USA) with three-dimensional adjustment capabilities. The cylindrical cathodic/detection reservoir (3.5 cm diameter×3 cm height, volume 20–25 ml) contained platinum wires (sealed with epoxy horizontally through the reservoir) for the counter electrode and the cathode for electrophoresis. The working electrode chip (2.1 cm×1.1 cm) was also sealed with epoxy horizontally half way through the reservoir while an Ag/AgCl reference electrode was placed vertically into the reservoir (Fig. 1A). The micromanipulator and a laboratory jack (to which the reservoir was solidly mounted on) were attached to a SA series breadboard (Newport, Fountain Valley, CA, USA) to prevent movement during alignment.

2.3. Interdigitated electrodes (IDE)

Interdigitated working electrodes of gold or platinum were fabricated by Windsor Scientific (Berkshire, UK), which contained an array of eight microband electrodes, each 2 mm in length by 10 μm in width with interspacing of 10 μm (Fig. 1B). The total electrode surface area was 16×10^4 μm^2 while the overall electrode array occupies an area of 2 mm×160 μm. The substrate (4 mm×4 mm, surface area) consisted of a layer of silicon nitride and thermal oxide on the silicon wafer with a thickness of 150 and 800 nm, respectively. A 200-nm silicon nitride passivation layer covered the active substrate surface. The depth or thickness of the gold and platinum layers were 330 and 180 nm, respectively, as determined by scanning probe microscopy (SPM, Dimension 3100, Nanoscope IV, Digital Instruments, Santa Barbara, CA, USA). Such results were close to the values provided by the manufacturer. The microelectrodes are covered by a passivation layer such that a 1 mm×1 mm section serves as the detection area. The eight pin positions of the IDE (external to the reservoir) were connected by a ribbon band to the eight leads of the multi-channel potentiostat (Model 1030, CH Instruments, Austin, TX, USA). The capillary outlet was aligned to the IDE using the micromanipulator with the aid of a surgical microscope (Luxo, Port Chester, NJ, USA) equipped with a charge-coupled device CCD camera. The capillary outlet was adjusted until it touched the IDE surface (evident by a slight bend in the capillary observed by

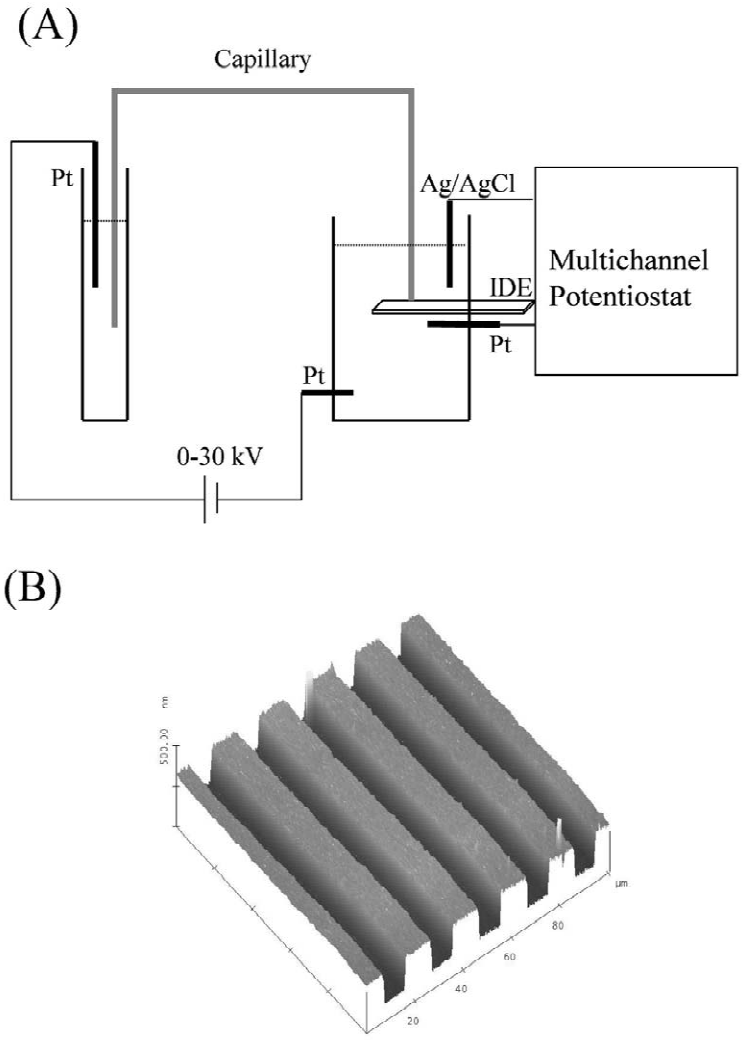

Fig. 1. (A) Schematic diagram of experimental set-up; (B) AFM image of platinum IDE—*x*, *y* scale of 100 μm, *z* scale of 200 nm.

camera) and it was then backed off 25–30 μm using the *z* control of the micromanipulator. It should be noted that the gold IDE surfaces were initially polished carefully with 1-μm diamond paste followed by 0.05-μm alumina slurry (Buehler) and washed with deionized water followed by sonication for 10 min in order to enhance the current signal. This initial cleaning step was not necessary for Pt IDEs as no improvement of the current signal was observed after cleaning.

2.4. Electrochemical measurements

Cyclic voltammograms (CV) of the eight electrodes in Tris-phosphate (prepared by the drop wise addition of 85% *ortho*-phosphoric acid into 50 m*M* Tris solution) buffer, pH 2.5 were monitored simultaneously between +1.2 V and −1.2 V at 0.5 V/s to condition the electrode. Typically, peaks were observed at −0.2 and −0.4 V (2–3 μA) during the oxidation wave and −0.6 V (2–3 μA) during the

reduction wave. The electrode surfaces were electrochemically cleaned for 90 s using this protocol between repeated runs to ensure good reproducibility. New capillaries were treated with 1 *M* NaOH for 15 min followed by extensive washing with deionized water and Tris-phosphate buffer.

2.5. Electrochemical detection and capillary alignment

The alignment of the working electrode surface with the capillary outlet with an outer diameter of 360 μm (covering the entire electrode surface) was easily performed. The oxidation of neurotransmitters (1 m*M*, diluted from 100 m*M* stock solutions in 50 m*M* Tris-phosphate buffer, pH 2.5) was detected at +1.1 V. The multi-channel potentiostat offered a simultaneous measurement for all eight positions and any slight adjustments could be aided by the micromanipulator until the maximum current was observed at the two central electrode positions. The electrode positions with respect to the channel leads from the multi-channel potentiostat were easily discerned by examining the IDE configuration under a stereomicroscope. For the monitoring of the recycling of neurotransmitters, one of the central electrodes (referred to as the detecting electrode) was poised at a reducing potential vs. the remaining seven positions (referred to as recycling electrodes) at oxidizing potentials. Optimal recycling conditions were determined by varying the oxidation and reduction potentials. The effect of ''microband gap'' on the current response of the recycling assay was conducted by using only two electrode positions with opposing potentials and moving the influencing electrode further away from the detecting position. The effect of the number of electrodes (from one up to seven) used for recycling, poised at the oxidizing potential compared to the detecting electrode, was also determined. The position of the detecting electrode on the array was switched from the center to the edges of the IDE surface to observe the impact on the current response. Calibration curves were established to estimate the detection limit for each of the enantiomers. The reproducibility of the migration time and the current signal in both peak height and peak area were determined for each enantiomer (1 m*M*).

3. Results and discussion

3.1. Optimal conditions for the electrochemical recycling scheme

Neurotransmitters often exhibit an endocrine and exocrine influence, effecting the production of hormones as well as playing a role in the diagnosis and treatment of diseases such as Parkinson's [34]. For instance, epinephrine (adrenaline) and norepinephrine, the two hormones secreted by the adrenal medulla, are released into the bloodstream in response to physical and mental stress to cause vasoconstriction [35]. Neurotransmitters have been separated by various CE methods utilizing different detection methods such as amperometric [36], laser-induced fluorescence [37], and mass spectrometry [38]. CE separation of neurotransmitter enantiomers has been achieved by adding various CD derivatives to running buffers [39]. The native β-CD is not able to chirally resolve epinephrine, norepinephrine and isoproterenol [40]. Only very poor resolution is obtained for the optical isomers of isoproterenol at relatively high concentrations of β-CD (20 m*M*). Of particular interest is DM-β-CD, which has been widely used to separate many enantiomers of neurotransmitters [6,10] as well as the enantiomers of drugs, both legal and illicit [41]. To our knowledge, there is no report on the chiral analysis of neurotransmitters using CE and IDE electrochemical detection.

Preliminary experiments with the racemic mixtures (equal concentration of each enantiomer) of the three neurotransmitters indicated that the sensitivity with respect to current response was about twofold higher using platinum IDEs compared to surface-cleaned gold IDEs, whether the experiment was conducted together with the separation capillary or simply in a stirred cell set-up without electrophoresis. However, there was no difference in the oxidative current for neurotransmitters when measurement was conducted at +1.1 V using a platinum or gold disk electrode (1.6 mm diameter). The slight

difference noted in the current response between the two different IDEs might be due to a difference in their methodologies of microfabrication and passivation layers (thickness and density). Since this information was proprietary, this subject could not be investigated further and platinum IDEs were used for all subsequent experiments. The distance between the capillary outlet and IDE was optimal at 25–30 μm. Interference from the separation potential was not noticed at this close distance since the current generated with the small diameter (20 μm) capillary was only 4 μA. With a gap distance below 15 μm, no signal was observed since there was no EOF in the capillary due to a very high back pressure. As expected, the oxidative current signal decreased with an increase in the gap distance; the current signals (compared to 25–30 μm) measured at 50, 100, and 200 μm were 58, 28, and 8%, respectively.

Tris-phosphate (50 m*M*) buffer, pH 2.5 was chosen as the separation buffer since at higher pH values chiral separation of the analytes would not be possible even with DM-β-CD. For each racemic mixture of the three neurotransmitters (1 m*M*), a CE recycling signal (−20 to −30 nA) in the absence of DM-β-CD was observed between 900 and 1050 s at the detecting electrode (0.0 V), while the seven recycling electrodes were set at +1.1 V. A similar result was observed using 25 m*M* MES buffer, pH 5.5, although the migration time (350 s) for the analytes was much shorter with no separation observed. A reduction potential of −0.4 V and an oxidation potential of +0.7 V at pH 5.5 was necessary to achieve similar results to those obtained at pH 2.5. The shift in the potential optimums was partly due to the different pH values and a positive potential shift (+0.55 to +0.75 V) at low pH was also observed when using the IDE in a stirred cell for the oxidative detection (no recycling) of the neurotransmitters. As expected from the electroosmotic flow (EOF), decreasing the Tris-phosphate concentration to 25 m*M* reduced the migration times for the analytes, but resulted in inferior separation.

As a test model, the three different racemic mixtures of the neurotransmitters (2 m*M*) were run under a chiral separation condition (50 m*M* Tris-phosphate pH 2.5, containing 15 m*M* DM-β-CD) similar to that previously reported [6] with the detecting reduction potential varying while the remaining seven adjacent recycling electrodes were set at +1.1 V. Under these separation and detection conditions, all six enantiomers (1 m*M*) could be optimized simultaneously. For the purpose of comparison, the maximum peak height current signals for the enantiomers of norepinephrine (20–21 nA), epinephrine (15–17 nA) and isoproterenol (11–12 nA) were normalized to 100%. The optimum current response occurred when the reducing potential was less than 0.0 V (Fig. 2A). With respect to the signal-to-noise ratio, 0.0 V was chosen as the reducing potential for the detecting electrode in all subsequent experiments. The current response curves were identical for each enantiomer of the three sets of

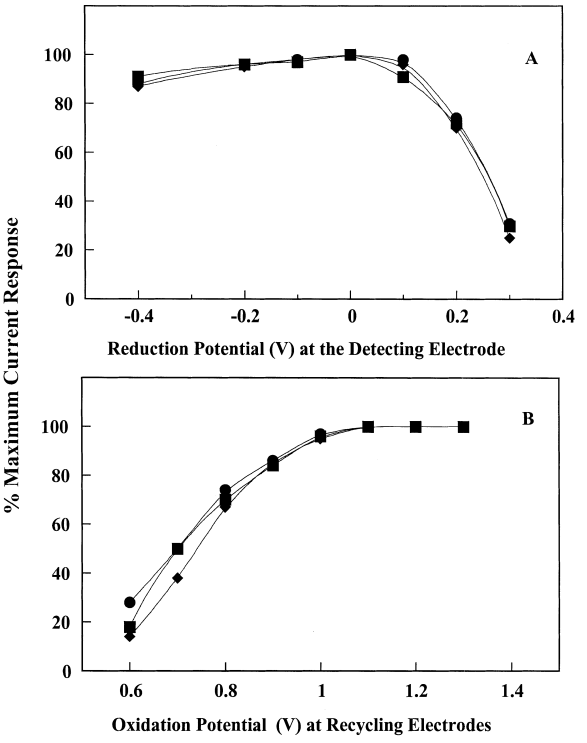

Fig. 2. (A) The effect of the reduction potential of the detecting electrode on the current response of the electrochemical recycling scheme with recycling electrodes at an oxidization potential of +1.1 V: epinephrine (■); isoproterenol (♦); and norepinephrine (●). Average for 1 m*M* of each enantiomer. (B) The effect of the oxidation potential of the seven recycling electrodes on the current response with the detecting electrode at a reduction potential of 0.0 V (symbols same as in A).

enantiomers, and the values plotted in Figs. 2 and 3 are the average for each pair.

The optimum oxidation potential of the recycling scheme was established as +1.1 V (Fig. 2B). In this case, the oxidizing potential of the seven recycling electrodes was varied while the reducing potential of the detecting electrode was fixed at 0.0 V. As the current signal began to decrease for all six enantiomers at +1.0 V, the potential of the recycling electrodes was set at +1.1 V. An enhancement of about twofold for the signal was observed at the two oxidizing electrode positions next to the detecting position when compared to control experiments, whereby all electrodes were oxidizing. With CE recycling schemes, the maximum current signal at the detecting electrode was only achieved when the oxidation potential at the recycling electrode was poised at high potentials (+1.1 V) instead of +0.6 V, often used for oxidation of neurotransmitters such as epinephrine (a half-wave potential of 0.51 V) with a single electrode system [42]. There could be three contributing factors for the shift in the oxidation potential. First, in stirred cell experiments without electrophoresis, the optimum oxidation potential shifted from +0.55 to +0.75 V due to the lower pH (2.5) employed for chiral separation as mentioned earlier. The second reason for a shift of +0.2 V (+0.75 to +0.95 V) was due to the recycling scheme compared to similar experiments in the absence of recycling. The last contributing factor was due to the effect of the high separation voltage (+25 kV). Positive potential shifts of +0.15 and +0.50 V have been previously reported [43], depending on separation capillary diameters of 25 μm (20 μm in our study) and 50 μm, respectively. In the CE recycling scheme for (ferrocenylmethyl)trimethylammonium perchlorate [31] with a half-wave potential of 0.44 V [42], a high oxidation potential (+1.0 V) was also necessary.

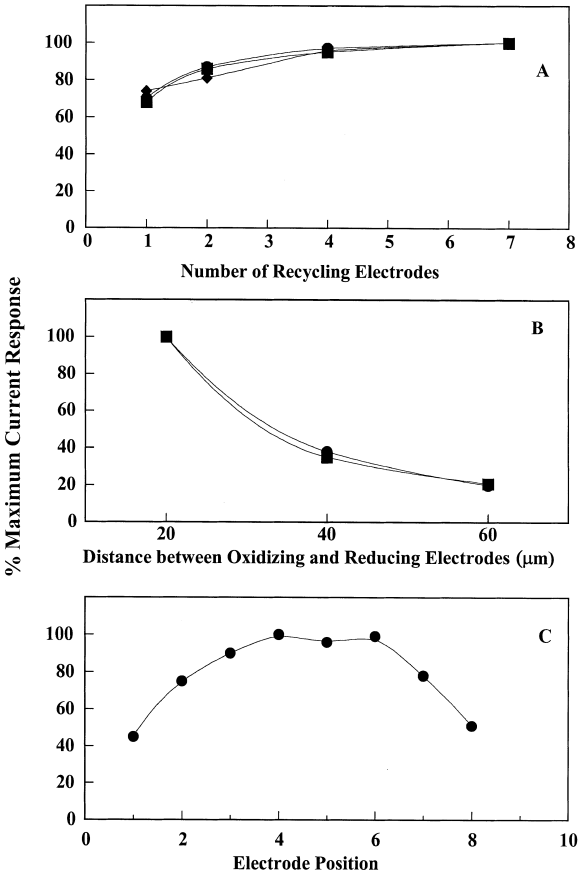

Fig. 3. (A) The effect of the number of recycling electrodes set at an oxidation potential of +1.1 V on the current response of the detecting electrode set at 0.0 V: epinephrine (■); isoproterenol (♦); and norepinephrine (●). Average for 1 m*M* of each enantiomer. (B) The effect of distance between an oxidizing electrode (+1.1 V) and the reducing detecting electrode (0.0 V) on the reductive current response (symbols same as in A). (C) The effect of positioning of the reductive electrode (0.0 V) of the recycling scheme on the current response with all remaining electrodes oxidizing (+1.1 V). Positions 4 and 5 are the central electrodes while positions 1 and 8 are at the edge of the platinum IDE.

3.2. Other effects on the recycling scheme

Fig. 3A illustrates the effect of the number of electrodes used in the recycling scheme on the current response of the detecting electrode. The maximum response was obtained for all six enantiomers when all seven recycling electrodes were poised at +1.1 V. However, as high as 80% of the maximum was still achieved with just two recycling electrodes. The effect of the distance between the oxidizing and reducing electrode positions on the current signal at the detecting electrode was shown in Fig. 3B. Utilizing just one oxidizing and one reducing electrode, the maximum peak height currents for the enantiomers of norepinephrine, epinephrine and isoproterenol were 14, 10 and 8 nA, respectively. For all six enantiomers, the current

response of the reducing detecting electrode decreased drastically as the distance was increased from 20 μm (adjacent electrode oxidizing) to 60 μm (three electrodes away). This influence of the gap distance between recycling electrodes has also been reported for stationary solution experiments [44]. This was of importance since it implied that further reduction of the gap distance between the recycling positions would further enhance the resulting signal to improve detectability and peak sharpness. The important aspect for the selection of the detecting electrode with respect to the other seven recycling electrodes was illustrated in Fig. 3C. When the electrode at either two extremities was used for detection, the current response was only ~40% of the signal obtained at the central detection position. In this series of experiments, the capillary position was not adjusted and the results were an average taken from all six enantiomers.

3.3. Chiral separation and analysis of neurotransmitters

Chiral separation was performed using a sample containing 1 m*M* of the three racemic mixtures of neurotransmitters in 50 m*M* Tris-phosphate buffer, pH 2.5 with different concentrations of DM-β-CD. Under these separation conditions, the three racemic mixtures were positively charged owing to the protonation of the basic nitrogen atoms and migrated towards the cathode [10]. Full protonation could be assumed since the pK_a values should be between 8 and 10. As expected, without DM-β-CD in the running buffer, only three peaks emerged between 900 and 1050 s (Fig. 4A). Spiking experiments confirmed that the migration order was norepinephrine, epinephrine, and isoproterenol. At 5 m*M* DM-β-CD, the two enantiomers of isoproterenol were well separated, while the enantiomers of epinephrine had just begun to split and norepinephrine still migrated as a single peak (Fig. 4B). Under this pH condition, DM-β-CD exhibited a neutral charge and migrated together with electroosmosis, and such results indicated that isoproterenol the most hydrophobic analyte formed the most stable complex with this chiral selector, followed by epinephrine, and norepinephrine. At 15 m*M* DM-β-CD, the enantiomers of both epinephrine and isoproterenol were well separated and those of norepinephrine had begun to split (Fig. 4C). Finally, baseline separation of the six enantiomers was achieved at 25 m*M* DM-β-CD (Fig. 4D). This value was higher than the optimal DM-β-CD (18 m*M*) obtained with on-line UV detection [6], which was likely due to the poorer resolution usually observed with end-column detection devices where

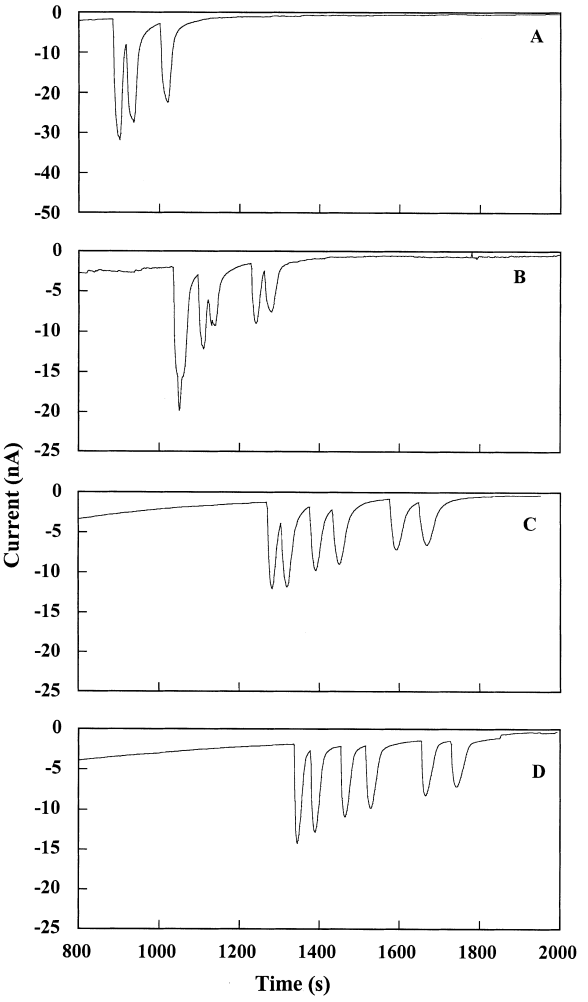

Fig. 4. The effect of heptakis(2,6,di-*O*-methyl)-β-cyclodextrin concentration in 50 m*M* Tris-phosphate, pH 2.5 buffer on the capillary electrophoretic separation of 1 m*M* racemic mixture of neurotransmitters: (A) 0 m*M*; (B) 5 m*M*; (C) 15 m*M*; and (D) 25 m*M*. The reduction potential of the detecting electrode was 0.0 V while the recycling electrodes had an oxidation potential of +1.1 V. Separation order: norepinephrine, epinephrine and isoproterenol. Note that the concentration of each enantiomer in D would be 0.5 m*M*.

diffusion of analytes occurred. The total peak areas of the enantiomer pairs in Fig. 4D matched the total area of the three single peaks (for both enantiomers under non-chiral separation conditions, Fig. 4A), which implied that the addition of DM-β-CD did not interfere with the recycling mechanism. The identification of the enantiomers was performed by spiking experiments using the same separation conditions as Fig. 4D. The (−) enantiomeric form of each neurotransmitter was always the first peak of each pair (figure not shown), consistent with results reported by Fanali [6]. Such behavior revealed that the (+) enantiomer formed a more stable diasteroomeric complex with DM-β-CD than its (−) enantiomer counterpart.

3.4. Collection efficiency and redox cycling

Under the optimal separation condition (25 m*M* DM-β-CD), there was no response for the racemic mixture of the neurotransmitters (2 m*M*) when all electrode positions were poised at 0.0 V (Fig. 5A). The low noise level of about 20–30 pA could be easily observed. The result was totally expected since the compounds were already in the reduced state and could not be detected at 0.0 V. Fig. 5B illustrates the current response at the central detecting electrode when all electrode positions were poised at +1.1 V with the two adjacent positions producing similar results. Switching the central position to 0.0 V (reducing electrode) creates a recycling scheme with the adjacent oxidizing electrode and utilizing just these two electrodes will mimic the dual electrode condition. The current response at the reducing electrode was 68–74% optimal (see Fig. 3A, one vs. seven recycling electrodes) while the current response at the oxidizing electrode increased 2.3–3.2-fold depending on the enantiomer. This amplification indicated that there was about two to three redox cycles similar to the number of redox cycles reported with IDRAs [32,33] and interdigitated array electrodes coupled to wall-jet cells [45,46], but unlike in the case of the dual electrode configuration [31] where redox cycling was not observed. The collection efficiency (ratio of reduction electrode current divided by the oxidation electrode current) was 62–67%, depending on the enantiomer. The collection efficiency was higher than those reported using the dual-electrode configuration [23,25,31] and similar to those reported for the interdigitated disk-ring array electrodes [32,33]. However, using IDRAs epinephrine was quite problematic with only 17% collection efficiency. With an applied potential of 0.0 V at the detecting position and +1.1 V at the remaining seven oxidizing positions, the maximum reducing current response was observed (Fig. 5C). With all eight electrodes in operation, the signal at the adjacent position (oxidizing) to the detecting (reducing) electrode dropped slightly (65%) compared to the dual electrode configuration such that the signal was amplified by 1.7–2.2-fold depending on the enantiomer (Fig. 5D). Increasing the number of oxidizing electrodes from one to seven increased the reduction current signal 1.6-fold such that the reduction current signal was 1.2-fold greater than the oxidation current signal at the adjacent electrode. The collection efficiency was not determined for the configuration using all eight electrodes since this terminology is normally applied to dual-electrode configurations where the number of oxidizing and reducing electrodes is equal.

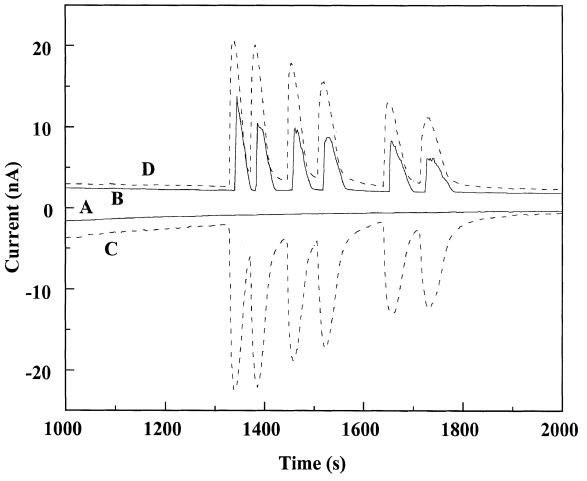

Fig. 5. Separation of 1 m*M* enantiomer in the presence of 50 m*M* tris-phosphate, pH 2.5, 25 m*M* heptakis(2,6,di-*O*-methyl)-β-cyclodextrin. (A) All eight electrodes with a reduction potential of 0.0 V; (B) all eight electrodes with an oxidation potential of +1.1 V; (C) detecting electrode position (0.0 V) with seven recycling positions at +1.1 V; (D) oxidizing electrode adjacent to detecting electrode position (0.0 V) with seven recycling positions at +1.1 V.

The number of redox cycles observed and the collection efficiency are very dependent upon a number of important factors, such as the separation voltage, capillary diameter, flow-rate, the capillary to electrode distance [33], and the gap distance between the recycling electrodes [44]. Decreasing the separation voltage or capillary diameter reduces the flow-rate and thus increases the collection efficiency and the number of redox cycles. The increase in redox cycles observed in comparison to the dual electrode configuration [31] using the 75-μm capillary (flow-rate of 600 nl/min) was likely due to the smaller capillary diameter (20 μm), which would result in a slower (nl/min) flow-rate, resulting in a longer residence time at the electrode surface. The low pH also decreased the electrophoretic mobility of the analyte and resulted in more time for recycling. Decreases in the gap distance between recycling electrodes also increased the collection efficiency and number of redox cycles, as previously explained with respect to Fig. 3B. Increasing the capillary to electrode distance increases the number of redox cycles and collection efficiency at the expense of the current value obtained so a compromise must be chosen [33]. For the present set-up in this study, increasing the gap between the capillary outlet and the electrode from 25 to 60 μm simply decreased the current signal by half without increasing the number of redox cycles. A series of experiments was also conducted using a 50-μm I.D. capillary, which yielded a reduction current signal of only 0.5-fold the oxidation current signal at the adjacent electrode and slight redox cycling (1.5×) was observed only for norepinephrine (data not shown).

Applying oxidizing (+1.1 V) and reducing (0.0 V) potentials to alternating microband fingers (figure not shown) did not increase the current detected at the reducing fingers (90–95%) in comparison to the described detector configuration. Using the alternating finger approach which would be similar to four dual electrodes in series, the currents at the oxidizing fingers were slightly higher (1.5-fold, indicating 3.5 redox cycles and the reduction current signal was only 0.75-fold the oxidation current signal at the adjacent electrode) than that with the described detector configuration, since the number of reducing electrodes adjacent to oxidizing electrodes has increased from one to two. Since the number of oxidizing and reducing electrodes are equal, a pseudo-collection efficiency of 75% can be estimated by dividing the total reducing current of all four reducing electrodes by the total oxidizing current of the remaining four electrodes. However, the main objective was to perform analysis at the reducing electrode to circumvent interferences and fouling, therefore, we focused on the detector configuration with one reducing electrode versus seven oxidizing electrodes.

The higher reduction current and amplification due to redox recycling would increase the detectability of the analytes using the detector configuration along with the multi-channel potentiostat for simultaneous control of all eight electrodes, in comparison to the dual electrode format. In addition to the amplification effect, the key beneficial feature of the scheme was the capability of detection of reducing compounds at a reducing potential to alleviate electroactive interferences and electrode fouling. A concurrent reduction/oxidation detection for each optical isomer also facilitated and ascertained peak assignment. Alignment was seldom needed throughout the course of measurements and the results obtained were highly reproducible as addressed later.

3.5. Electrophoretic analysis of neurotransmitters using the recycling scheme

Experiments were conducted using the optimal conditions for the recycling scheme, and statistical analysis was performed at a 95% confidence interval with a sample size of 10 (Table 1). A good reproducibility (0.6–0.7%) of the migration time for all six enantiomers was observed. The reproducibility of the peak height and peak area current signals for 10 repeated injections (1 m*M*) of the six enantiomers was within 2.5–3.5%. A range of concentrations (0.005–4 m*M*) was analyzed and the signal was linear up to 4 m*M* in the case of epinephrine and isoproterenol and 2 m*M* in the case of norepinephrine enantiomers, which were not quite as well separated. Notice that linearity for all six enantiomers was only observed in peak area mode and not in peak height mode. For example, as shown in Table 1 the peak height at 1 m*M* ranged from 10 to 20 nA depending on the enantiomer, whereas at 0.025 m*M* the range was 0.4–0.5 nA. The correlation coefficient (R^2) was 0.999 for all six enantiomers

Table 1
Statistics for neurotransmitter enantiomers using the recycling scheme

Enantiomer	Migration time (s)	Reproducibility (1 m*M*)		Sensitivity peak area (μC/m*M*)
		Peak height (nA)	Peak area (μC)	
(*R*)-(−)-Norepinephrine	1346±8	−21.0±0.4	0.53±0.01	0.49±0.01
(*S*)-(+)-Norepinephrine	1391±8	−20.2±0.3	0.57±0.01	0.58±0.01
(*R*)-(−)-Epinephrine	1464±9	−17.2±0.2	0.55±0.02	0.52±0.01
(*S*)-(+)-Epinephrine	1529±10	−15.2±0.3	0.54±0.01	0.54±0.01
(*R*)-(−)-Isoproterenol	1661±10	−11.9±0.3	0.44±0.01	0.41±0.01
(*S*)-(+)-Isoproterenol	1740±11	−10.7±0.1	0.45±0.01	0.44±0.01

Statistics were performed at a 95% confidence interval with a sample size of 10 with the exception of sensitivity (n=14).

and their detection limits were determined to be 5 μ*M* (S/N=3), since the typical noise value observed was 20–30 pA (Fig. 5A).

4. Conclusions

An array of ‘‘microband’’ interdigitated electrodes has been successfully demonstrated with cyclodextrin-modified capillary electrophoresis to facilitate the analysis of neurotransmitter enantiomers. Besides the ease of the capillary-electrode alignment to improve reproducibility, a scheme has been developed to improve the detection sensitivity, based on the pseudo-reversibility of such target analytes with respect to oxidation and reduction. However, the main feature of this detection scheme was the capability of detecting reduced compounds at a reducing potential instead of oxidation to circumvent electrode fouling and electroactive interferences. This scheme also facilitated and ascertained peak identification, since interfering compounds were unlikely to have the exact same oxidative/reductive characteristics. If necessary, interdigitated electrodes with submicrosized bands and gaps (less than a few microns) can be microfabricated to improve sensitivity and speed of analysis. Sample injection by pressure or vacuum is also expected to increase the separation efficiency as well as detection sensitivity. This detection scheme is also compatible with a high throughput capillary electrophoresis system, an emerging technology, to perform parallel separation of enantiomers with less time and sample consumption. In view of this, CE equipped with microfabricated interdigitated electrodes might become a powerful tool in chiral separation to achieve good sensitivity without resorting to lasers, mass spectrometry or other expensive instrumentation.

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