

## Determination of dopamine in rat less differentiated pheochromocytoma cells by capillary electrophoresis with a palladium nanoparticles microdisk electrode

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A simple method was described for the direct detection of dopamine (DA) in single rat pheochromocytoma cells (PC12 cells) by capillary electrophoresis with a palladium nanoparticles modified carbon fiber microdisk electrode (PdNPsCFME). The Pd nanoparticles could be electrodeposited on the surface of the carbon fiber microdisk electrode (CFME) with a simple pretreatment procedure. The PdNPsCFME provided an enhanced effective electrode surface and high catalytic activity toward dopamine compared to traditional CFME. Under optimized detection conditions, dopamine responded linearly from the range of 0.2  $\mu\text{M}$  to 2  $\mu\text{M}$  and 2  $\mu\text{M}$  to 0.1 mM with correlation coefficients of 0.9979 and 0.9990. The concentration detection limit was 0.1  $\mu\text{M}$  ( $S/N = 3$ ). The relative standard deviations of the migration time and peak current were 1.4% and 3.5%, respectively. This method had been successfully applied to determine DA in single PC12 cells and the amount of DA in ten PC12 cells ranged from 1.2 to 6.3 fmol. The above results demonstrated that CE coupled with PdNPsCFME is convenient, sensitive, and will become an attractive alternative method for single-cell analysis.

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### Introduction

Dopamine (DA) is one of the excitatory neurotransmitters that play a crucial role in the functioning of central nervous, renal and hormonal systems. Abnormal levels of DA are related to neurological disorders such as Parkinson's,<sup>1</sup> Alzheimer's disease<sup>2</sup> and Schizophrenia.<sup>3</sup> It has been also suggested that DA plays a role in drug addiction<sup>4</sup> and some manifestations of HIV.<sup>5</sup> Therefore, one of the main tasks of neurochemistry is the development of a simple and quick method for dopamine detection and determination.

Various analytical methods that have been reported for the determination of DA include laser-induced fluorescence detection,<sup>6</sup> electrochemiluminescence<sup>7,8</sup> and electrochemical detection (ECD).<sup>9–12</sup> Although analysis of the DA by the above mentioned methods is appropriate, most of those methods require expensive equipment or complicated and time-consuming pretreatment procedures, such as derivatization. Thus, relatively simple, rapid and inexpensive analytical methods for the determination of DA would be of considerable interest and benefit in their applications.

Electrochemical techniques offer alternatives to those above-mentioned methods, rapid and sensitive procedures, the apparatus of which can be readily miniaturized and produced at relatively low cost. Examples of methods of analyses for the DA include the use of various types of film modified electrodes: graphene,<sup>9</sup> polymerized melanin<sup>10</sup> and metal nanoparticles<sup>11,12</sup> *etc.* Metal nanoparticles have attracted much more attention in electroanalysis because of their unusual physical and chemical properties.<sup>13</sup> Metal nanoparticles modified electrodes, especially those of noble metal nanoparticles, usually exhibit high electrocatalytic activities towards the compounds which have sluggish redox process at bare electrodes. Therefore, metal nanoparticles have been used in electrocatalytic reactions to facilitate heterogeneous electron transfer and increase analytical sensitivity for the determination of DA.<sup>14</sup> For example, Zhang *et al.* prepared a gold nanoparticles modified electrode and applied it to the electrocatalytic oxidation of ascorbic acid (AA) in the presence of DA.<sup>11</sup> Palladium (Pd) nanoparticles modified glassy carbon electrode was fabricated to determine dopamine in injection solutions.<sup>12</sup> Pd nanoparticle-loaded carbon nanofibers electrode displayed excellent electrochemical catalytic activities towards dopamine.<sup>15</sup>

Capillary electrophoresis (CE) has been widely applied for the analysis of single cells because of its small sample size of only nanoliters to femtoliters, high separation efficiency, short analysis time, and biocompatible environments. Electrochemical detection coupled to capillary electrophoresis can typically determine analytes present at femto-to attomole levels.

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Developments of microelectrodes fabrication techniques and different electrode material can improve both the sensitivity and selectivity, which has facilitated the widespread application of amperometric detection in single cell analysis. Up to now, carbon fiber microelectrodes have usually been employed for single cell assays to determine ascorbic acid,<sup>16</sup> histamine,<sup>17</sup> amino acids,<sup>18</sup> diclofenac sodium,<sup>19</sup> lactate dehydrogenase,<sup>20</sup> 5-hydroxytryptamine<sup>21</sup> and dopamine<sup>21,22</sup> *et al.* Ewing's group detected 5-hydroxytryptamine and dopamine in cytoplasmic injection from snail neuron through 2 and 5  $\mu\text{m}$  diameter capillary with a 5  $\mu\text{m}$  diameter carbon fibers etched to smaller diameters.<sup>21</sup> The operations of cytoplasmic injection and electrode fabrication were extremely complex. Cheng's group determined dopamine in single rat pheochromocytoma cell in 25  $\mu\text{m}$  diameter capillary with an 8  $\mu\text{m}$  diameter carbon fiber electrode.<sup>22</sup> The end of capillary was etched in 40% hydrofluoric acid to be inserted in the carbon fiber electrode. These methods were nevertheless time-consuming, labour-extensive and one-off. It is necessary to develop simpler fabrication of high sensitive working electrode of CE-ECD and more convenient operation to detect DA in individual cell analysis.

In this work, PdNPs were electrodeposited onto the carbon fiber microdisk electrodes directly. The modification of PdNPs increased the surface areas of working electrode with enhanced electron transport characteristics. Therefore, compared with the single carbon fiber microelectrodes, experimental results showed that the PdNPs modified microdisk electrode (PdNPsCFME) used in CE-ECD to determine DA had the properties of simple fabrication, large electrochemical response and good catalytic activity. To our knowledge, PdNPs modified electrode has never been used as the detector for CE-ECD. Dopamine in single PC12 cells was successfully determined without any pretreatment.

## Experimental

### Apparatus and equipment

All electropherograms were obtained on a self-assembled CE system. Briefly, a reversible high-voltage power supply (Instrument Company, Shandong Normal University, China) provided a variable voltage of 0.0–30.0 kV across the capillary, with the outlet of the capillary at ground potential. A 37 cm fused-silica capillary (25  $\mu\text{m}$  I.D., 375  $\mu\text{m}$  O.D. Yongnian Optical Conductive Fiber Plant, China) was placed between the two buffer reservoirs. The positive pole of high voltage was applied at the injection end, while the negative pole was held at ground potential by a coiled Pt wire placed at the bottom of the reservoir containing the ECD cell. Separations were carried out at an applied voltage of 14.0 kV. The detection cell was shielded in a metal box to reduce external disturbance. A CHI832b Electrochemical Analyzer (Shanghai Chenhua Instrument Company, China) was used to determine the concentration of dopamine with electrochemical detection (ECD). ECD was carried out with a three-electrode system consisted of a PdNPsCFME working electrode as the detector, a saturated calomel electrode (SCE) as the reference electrode, and a coiled Pt wire placed at the bottom of the cell as the auxiliary electrode. Inverted biological

microscope (Chongqing Optical & Electrical Instrument Co., Ltd. Chongqing, China) and stereomicroscope (Nanjing Jiangnan Novel Optics Co., Ltd. Jiangsu, China) were used in the experiment. The electrode surface was characterized by using an S-4800 ultra-high resolution scanning electron microscopy (Hitachi, Japan).

### Reagents and solutions

Dopamine (DA), ascorbic acid (AA) was purchased from Sigma Chemical Company (USA). Tryptophane (Try), uric acid (UA), cysteine (Cys) was from Amresco (USA) and  $\text{K}_2\text{PdCl}_4$  from Shanghai Bao Man biotechnology Limited Company. Other chemicals were of analytical reagent grade and used without further purification. The running buffer was 25 mM pH 7.4 phosphate buffer solution (PB). DA stock solution (10 mM) was diluted to the desired concentration with running buffer and freshly prepared every week. Cells were suspended in a balanced phosphate buffered saline (PBS) composed of 0.162 M  $\text{NaH}_2\text{PO}_4$  and 0.2 M  $\text{Na}_2\text{HPO}_4$  (pH 7.4). All solutions were stored at 4 °C in darkness and were filtered through 0.22  $\mu\text{m}$  membrane filter before use. Double-distilled water was used throughout.

### Fabrication of Pd nanoparticles modified electrode

A wisp of carbon fiber (6  $\mu\text{m}$  diameter, Shandong University Carbon Fiber Technology Research Center, China) was carefully inserted into a fused silica capillary (*ca.* 250  $\mu\text{m}$  I.D., 375  $\mu\text{m}$  O.D., 4.0 cm in length), and imbued with ethyl  $\alpha$ -cyanoacrylate adhesive. The other end of the carbon fiber was circled on a copper wire and smeared with Ag electric adhesive (Sino-platinum Metals Co., Ltd, China). It was dried in the oven at 70 °C for 30 min, and the joint of the capillary and the carbon fiber was inserted and fixed into a glass tube (400  $\mu\text{m}$  I.D., 700  $\mu\text{m}$  O.D., 4 cm in length) by using ethyl  $\alpha$ -cyanoacrylate adhesive. The carbon fiber microdisk electrode (CFME) was well prepared.

Prior to the modification, the surface of CFME was carefully polished on metallographic sandpaper and then sonicated successively in water, ethanol and water for 5 min. According to the method previously reported by Chen and co-workers,<sup>12</sup> PdNPs were electrodeposited on the surface of CFME by applying a repetitive potential scan between 1.2 and  $-0.2$  V (at the scan rate of  $0.1 \text{ V s}^{-1}$ ) for 3 circles in a solution contained 2 mM  $\text{K}_2\text{PdCl}_4$  and 0.5 M  $\text{H}_2\text{SO}_4$ . The PdNPs electrode was then removed and washed thoroughly with water for use.

Compared with the CFMEs previously used,<sup>17–22</sup> the CFME used in this study was friendlier to the environment because of free mercury. Moreover, compared with the single carbon fiber microelectrode used by Cheng's group<sup>22</sup> to determine DA in single rat pheochromocytoma cell, the PdNPsCFME was easily fabricated. And the small size of the new PdNPsCFME made it flexible and convenient to be used in the narrow detection cell.

### CE procedure and amperometric detection apparatus

CE was performed on the self-assembled capillary electrophoresis system. The CE separations were conducted in a 37 cm fused-silica capillary (25  $\mu\text{m}$  I.D., 375  $\mu\text{m}$  O.D.) at room temperature. The injection end of the capillary was immersed in

electrophoresis buffer reservoir. The other end of the capillary (ca. 5 mm of the polyimide-coating capillary was removed by burning) was immersed in the other buffer reservoir and adjusted to the same horizontal line as the micro-biosensor under the guidance of a three-dimensional micromanipulator. In order to see the collimation and space between micro-biosensor and the end of the capillary, a microscope with a magnification of  $40\times$  was used. When the equipment was fixed well, separation voltage of 14.0 kV separation field was applied across the capillary and the detection potential of 0.80 V was applied at the PdNPsCFME and the electropherogram was recorded. After the electroosmotic current reached a constant value, the electromigration injection was carried out. An injection voltage of 5.0 kV was applied for 10 s to transport the dopamine solution into the capillary tip. Then the capillary was put carefully to the CE electrophoresis buffer and the separation voltage was turned back to 14.0 kV. The capillaries were rinsed with 0.1 M NaOH solution, double distilled water and the corresponding buffer solutions for 5 min respectively by means of a syringe before run. Amperometric measurements in three-electrode mode were done using an Electrochemical Analyzer.

### Cell culture

Rat less differentiated pheochromocytoma cell lines (PC12 cells) were obtained from the Chinese Academy of Sciences in Shanghai, cultured in the proper culture medium. The cells were incubated at  $37\text{ }^{\circ}\text{C}$  under a 5%  $\text{CO}_2$  incubator (MCO-15AC, SANYO) in complete medium (Roswell Park Memorial Institute Medium 1640 (HyClone SH30809.01) supplemented with 10% heat-inactivated fetal bovine serum,  $100\text{ U mL}^{-1}$ , penicillin, and  $100\text{ }\mu\text{g mL}^{-1}$  streptomycin). Before analysis, PC12 cells in culture medium were washed three times and suspended with PBS.

### Single cell injection

PC12 cells suspended in PBS were added to self-made reservoirs. A 3 kV voltage was applied for about 5–10 s to transport the whole cell into the etched capillary tip until the cell has been immobilized to the inner of capillary under the microscope. The injection end of the capillary was removed and immersed into the CE electrophoresis buffer. Then 5 kV injection voltage was applied for about 5 s to lysis. After that, the separation voltage of 14.0 kV was applied to carry out an electrophoretic separation.

## Results and discussion

### SEM images of CFME and PdNPsCFME

The morphologies of CFME and PdNPsCFME were characterized by scanning electron microscopy (SEM). Fig. 1A showed the SEM image of CFME whose surface was smooth. The SEM image of the PdNPsCFME (Fig. 1B) showed that the Pd nanoparticles were uniformly dispersed on the electrode surface. During the electrochemical deposition process, few Pd nanoparticles were gathered to form as a group of nanoparticles which had been noticed in the SEM. The diameters of the electrodeposited nano Pd particles had been found to be less than 100 nm. Finally, the

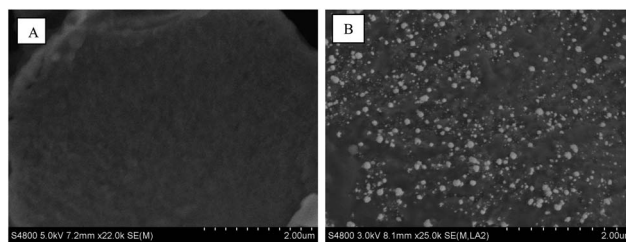


Fig. 1 SEM images of the surface of CFME (A) and PdNPsCFME (B).

SEM analysis result authenticated the deposition of Pd nanoparticles on the electrode surface.<sup>12</sup>

### Electrocatalytic oxidation of DA

It has been shown that gold nanoparticles (AuNPsME) and PdNPs have the electrocatalytic effects to DA.<sup>15,23</sup> The electrocatalytic oxidations of DA had been examined on PdNPsCFME, AuNPsCFME and CFME by cyclic voltammetry. The DA oxidation at the CFME took place at 0.218 V, but for the PdNPsCFME, the oxidation potential shifted negatively at 0.174 V (see Fig. 2) with a slightly enhanced anodic peak current. It might be because the size and number of PdNPs on the surface of the electrode were small (shown in Fig. 1B). With the change of electrodeposition, the size and number of PdNPs increased and consequently the anodic peak current enhanced obviously. This observation showed that palladium nanoparticles demonstrated high electrocatalytic activities towards DA. Although the cyclic voltammetric behavior of AuNPsME was similar with that of PdNPsCFME (not shown), the Pd compounds cost less. Therefore,  $\text{K}_2\text{PdCl}_4$  was used to fabricate the nanoparticles.

### Effect of electrodeposition conditions

The size and number of the nanoparticles influenced the electrocatalytic oxidations of the electrode and hence the sensitivity. The effect of electrodeposition conditions was investigated, respectively.

The effect of CV electrodeposition potential was investigated by varying the potential range. When  $-0.2$  to  $1.2\text{ V}$  was applied,

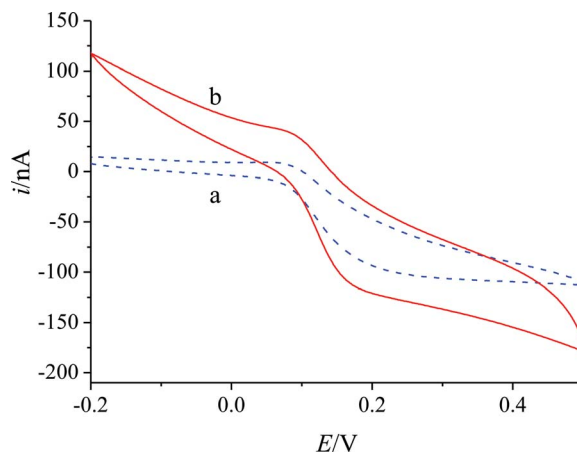


Fig. 2 Cyclic voltammetric behavior of 1 mM DA at CFME (a) and PdNPsCFME (b) in pH 7.4 0.2 M phosphate buffer. Scanning rate:  $50\text{ mV s}^{-1}$ .

it was found that the electrophoresis peak current of DA became largest. In this experiment,  $-0.2$  to  $1.2$  V was selected as the optimum electrodeposition potential.

The effect of electrodeposition cycles was investigated by varying from 1 cycle to 6 cycles. It was found that the peak current enhanced with the increase of the electrodeposition cycles before 3 cycles. When 3 cycles were applied, the peak current became largest. Then the peak current decreases due to the increase of the size of PdNPs. In addition, with the increase of the cycles, the peak shape appeared tailing area. Therefore, 3 cycles were selected in consequent experiments in order to obtain the PdNPs.

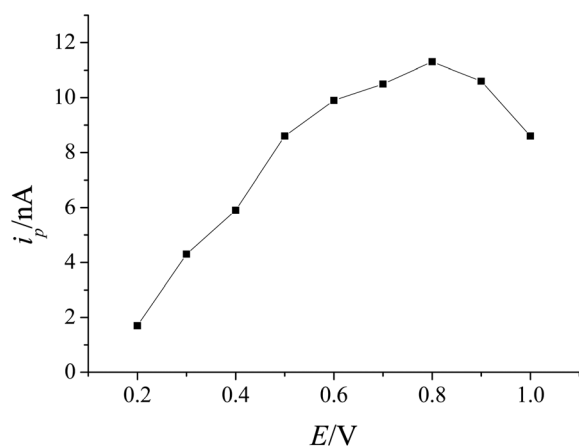
The concentrations of  $K_2PdCl_4$  have an important effect on the peak current. The effect of  $K_2PdCl_4$  concentration was investigated by varying the concentration ( $0.5$  mM;  $1$  mM;  $2$  mM;  $3$  mM;  $4$  mM). The peak current increased sharply from concentration  $0.1$  mM to  $2$  mM, and then the peak current decreased slowly from  $2$  mM to  $4$  mM. Moreover, when the concentrations of the  $K_2PdCl_4$  were higher than  $2$  mM, the PdNPs were aggregated seriously when observed by SEM. Therefore, the optimized concentration for this work was  $2$  mM.

### Effect of detection potential

The applied detection potential affects the detection sensitivity, detection limit, and baseline noise greatly. So the effect of detection potential on the determination of dopamine ranging from  $0.2$  V to  $1.0$  V was investigated (Fig. 3). When the detection potential was lower than  $0.8$  V, the peak current increased accordingly, and then decreased slowly. Moreover, the background current increased along with the increase of detection potential. Therefore, the background current is not too high, the stability and reproducibility are better and the  $S/N$  ratio is the highest when the detection potential of  $0.8$  V was selected.

### Effect of separation voltage and injection time

The applied separation voltage controls the electro-osmotic flow and the separation process. So it affects the peak height,



**Fig. 3** Hydrodynamic voltammogram for  $0.1$  mM dopamine. Fused-silica capillary:  $25$   $\mu$ m I.D./ $37$  cm; running buffer:  $25$  mM PB (pH  $7.4$ ); electrodeposition conditions:  $-0.2$  to  $1.2$  V/ $3$  cycles in  $2$  mM  $K_2PdCl_4$  and  $0.5$  M  $H_2SO_4$ ; separation voltage:  $14$  kV; injection time:  $5.0$  kV/ $10$  s.

**Table 1** Influence of separation voltage with  $0.1$  mM dopamine<sup>a</sup>

$E$ /kV	$i_p$ /nA	$t_m$ /s	$W_{1/2}$ /s	$N$
6	5.8	463	8.5	16 437
8	7.2	338	6.2	16 465
10	8.6	259	4.7	16 823
12	10.1	214	3.8	17 570
14	11.0	173	3.0	18 423
16	11.9	144	2.8	14 653

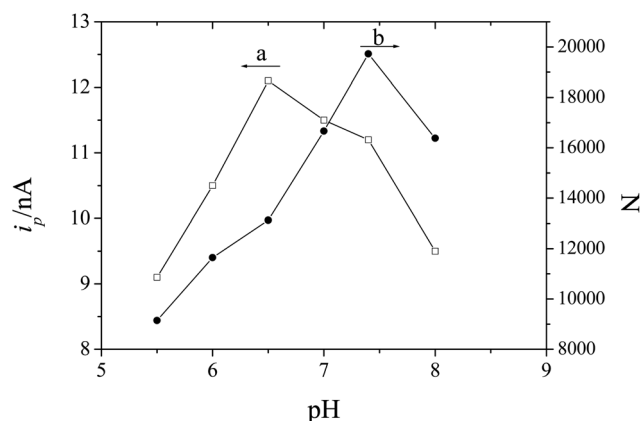
<sup>a</sup> Detection potential:  $0.8$  V. Other conditions as in Fig. 3.

migration time and the width at the peak half-height ( $W_{1/2}$ ). To evaluate the effect of the separation voltage ( $E$ ), we compared the peak current ( $i_p$ ), migration time ( $t_m$ ),  $W_{1/2}$  and theoretical plate number ( $N$ ) of dopamine over the range of  $6.0$ – $16.0$  kV (Table 1). As can be observed if higher separation voltage was applied, larger current, shorter running time and narrower peak were achieved. In addition, when higher separation voltage was applied, the noisy baselines became larger. When  $16$  kV was applied, the peak current became largest, but  $N$  turned smaller. As a compromise between the analysis time, the peak current and theoretical plate number,  $14$  kV was fixed as the optimum separation voltage.

The effect of the injection time was studied in the range from  $5$  s to  $20$  s with an injection voltage of  $5$  kV. When the time of injection was increased, the peak current and peak width increased. Shorter injection time would make the peak current weaker, while longer injection time would broaden the width of peak severely. In this experiment,  $10$  s (at an injection voltage of  $5$  kV) was selected as the optimum injection time.

### Effect of the pH and concentration of the buffer

The concentration and pH of the running buffer have an important effect on the surface characteristics of the fused-silica capillary and the effective electric charge of the ion. The pH of the  $25$  mM PB buffer on  $i_p$  (curve a) and  $N$  (curve b) was shown in Fig. 4. As it was found, the peak current increased dramatically when the value of pH was less than  $6.5$ , and then it



**Fig. 4** Effect of acidity on peak current (a) and theoretical plate number (b). Other conditions as in Table 1.



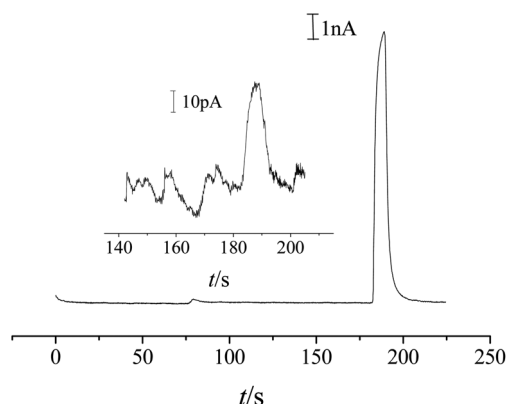
decreased gradually. When pH 7.4, the theoretical plate number became maximum value and  $W_{1/2}$  reached a minimum value. For a satisfactory detection the optimum pH of buffer was selected at 7.4.

The effect of the buffer concentration ( $C_b$ ) on  $t_m$ ,  $i_p$ ,  $W_{1/2}$ , and  $N$ , was studied in the range from 25 mM to 75 mM. When the concentration of PB increased,  $i_p$  decreased dramatically,  $t_m$  and  $W_{1/2}$  increased gradually. When 25 mM was used,  $N$  became maximum value. So 25 mM is suitable for the detection of dopamine.

### Linearity, limit of detection and reproducibility

The CE separations were conducted in a 37 cm fused-silica capillary at room temperature with a separation voltage of 14.0 kV. The capillary was adjusted to the same horizontal line as the electrode with the aid of stereomicroscope. An injection voltage of 5.0 kV was applied for 10 s to transport the dopamine solution into the capillary tip. The electropherogram was recorded by CHI832B electrochemical workstation. Fig. 5 showed the representative electropherogram of 0.10 mM and 0.1  $\mu$ M DA solution under the optimized conditions.

An investigation was performed to determine the linear range, limit of detection (LOD), and reproducibility, and the results are shown in Table 2. Under the optimized detection conditions, there is a linear relation of the peak area with the



**Fig. 5** The electropherogram of the standard solution of 0.10 mM and 0.1  $\mu$ M (insert) dopamine. Other conditions as in Table 1.

**Table 2** Analytical performance of the proposed method ( $n = 3$ )<sup>a</sup>

Linearity range	Regression equation	<i>R</i>	RSD <sup>b</sup> (%), $t_m$	RSD <sup>b</sup> (%), $A_p$	LODs
1 $\mu$ M to 0.1 mM	$A_p$ (nC) = $-0.6764 + 763.2C$ (mM) $A_p$ (nC) = $-0.6764 + 0.2544n$ (fmol)	0.9990	1.4	3.5	0.1 $\mu$ M
0.2–2 $\mu$ M	$A_p$ (nC) = $0.02869 + 0.5756C$ ( $\mu$ M) $A_p$ (nC) = $0.02869 + 0.1919n$ (fmol)	0.9979			0.34 fmol

<sup>a</sup> Other conditions as in Table 1. <sup>b</sup>  $n = 10$ .

**Table 3** Comparison of different modified electrodes for DA determination

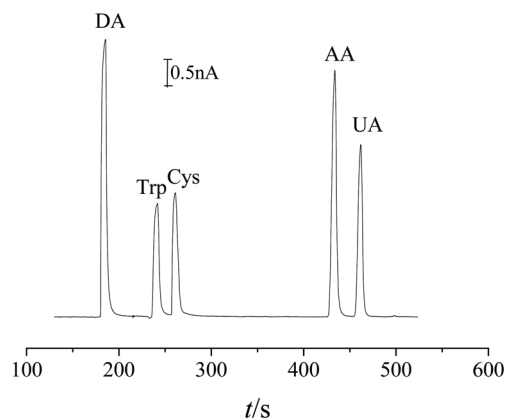
Electrodes	Linearity range ( $\mu$ M)	LODs ( $\mu$ M)	Ref.
Graphene modified electrodes <sup>a</sup>	4–100 $\mu$ M	2.64	9
PdNPs modified glassy carbon electrodes <sup>a</sup>	8–88 $\mu$ M	—	12
Sulfhydryl-terminated monolayer and the gold nanoparticles-immobilized glassy carbon electrodes <sup>a</sup>	6.5–145 $\mu$ M	2.8	23
Melanin-type polymer-modified carbon electrodes/CE <sup>b</sup>	1–25 $\mu$ M	0.9	10
Sputtered gold electrodes/CE <sup>b</sup>	20 $\mu$ M to 0.2 mM	0.1	24
Carbon paste electrode/MCE <sup>c</sup>	4–500 $\mu$ M	1.2	25
PdNPsCFMEs/CE <sup>b</sup>	0.2 $\mu$ M to 0.1 mM	0.1	This paper

<sup>a</sup> Voltammetry. <sup>b</sup> Capillary electrophoresis. <sup>c</sup> Microchip capillary electrophoresis.

concentration and the mass of DA between 1  $\mu$ M to 0.1 mM and 0.2  $\mu$ M to 2  $\mu$ M. The number of replications of each concentration level was three. The mean values were used to get the regression equation. When ten consecutive injections of 0.1 mM dopamine were performed in the analytical procedures, the relative standard deviation (RSD) of  $t_m$  and peak area ( $A_p$ ) were 1.4% and 3.5% respectively, so the stability of PdNPsCFME was acceptable in the above operation conditions. The detection limit for dopamine was found to be 0.1  $\mu$ M ( $S/N = 3$ ). Since the injection volume was 3.4 nL, the mass detection limit was 0.34 fmol. The sensitivity was amplified under similar detection condition, compared with the bare carbon fiber electrode, which was due to the fact that the PdNPsCFME provided an enhanced effective electrode surface and high catalytic activity toward DA. The baseline noise did not increase obviously although the area of electrode was enhanced. Therefore, the ratio of signal to noise increased. Compared with other methods, the proposed new method provided comparable linear range and detection limit with a simple electrode preparation procedure as listed in Table 3. Therefore, the PdNPsCFME exhibits an excellent reproducibility, high sensitivity and good stability for determination of dopamine by CE-ECD.

### Interference study

It is well known that some electroactive substance, such as ascorbic acid (AA), L-cysteine (Cys), tryptophan (Trp) and uric acid (UA) often coexist with dopamine in the biological matrix. To investigate the feasibility of the detection system for analysis of a biological sample, the electropherogram of the mixture of dopamine and these four analyses was showed in Fig. 6. The result shows that these substances cannot give interference effects, because their peak migration times are different with dopamine.



**Fig. 6** The electropherogram of the standard mixture of DA (50  $\mu\text{M}$ ), Trp (100  $\mu\text{M}$ ), Cys (100  $\mu\text{M}$ ), AA (200  $\mu\text{M}$ ), UA (100  $\mu\text{M}$ ). Other conditions as in Table 1.

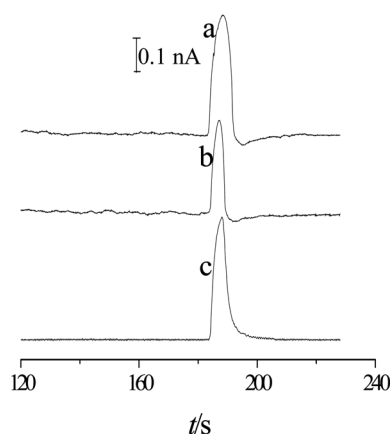
### Quantitative determination of dopamine in PC12 cells extract

The electrophoresis responses of the rat less differentiated pheochromocytoma cells (PC12 cells) extract are shown in Fig. 7 curve a. The only peak, eluting at about 190 s, can be identified as corresponding to DA in the cell extract on the basis of the migration time, as compared with the migration time of DA in the electropherogram shown in Fig. 7 curve c. The concentration of DA in the PC12 cells extract obtained by the standard calibration method is 1.6  $\mu\text{M}$ . Since the injection volume was 3.4 nL and the cell concentration in the PC12 cell extract was  $3.3 \times 10^5$  cell per mL, the mean mass of DA in a PC12 cell was calculated to be 4.9 fmol per cell.

### Individual cell analysis

Instead of 0.1% SDS was used to lysis the PC12 cells by Cheng's group,<sup>22</sup> the CE electrophoresis buffer was applied with 5 s/5 kV injection to lysis. Two reasons might be due to the anisotonic solution and the higher electric field (380 V  $\text{cm}^{-1}$ ) in CE. This made the operation of individual cell analysis more simplified.

The electropherograms of the contents of an individual PC12 cell are shown in Fig. 7, curve b. The migration time of the peaks



**Fig. 7** The electropherogram of cells extract (a), an individual PC12 cell (b) and 2  $\mu\text{M}$  DA (c). Other conditions as in Table 1.

**Table 4** Migration time ( $t_m$ ), peak area ( $A_p$ ), and amount of DA ( $q$ ) in single PC12 cells

Cell no.	$t_m/\text{s}$	$A_p/\text{nA}$	$q/\text{fmol}$
1	186	1.24	6.3
2	191	1.01	5.1
3	189	0.64	3.2
4	192	1.14	5.8
5	190	0.83	4.2
6	181	0.25	1.2
7	190	0.70	3.5
8	188	0.56	2.8
9	194	0.32	1.5
10	185	1.06	5.4

eluted at 190 s is with the same migration time as that of the peak of DA shown in Fig. 7 curve c. Thus, the peak can be identified as the peak of DA in an individual PC12 cell. The reproducible peak intensity, together with the large linear dynamic range for standard DA made it suitable to use external standardization for the quantification of DA in a PC12 cell. The results of analysis for ten single PC12 cells are listed in Table 4, which shows that the amounts of DA in single cells differ from cell to cell. The amounts of DA determined in ten cells range from 1.2 to 6.3 fmol per cell. The average content of DA in single PC12 cells found to be  $3.9 \pm 1.7$  fmol was agreed fairly well with the value of 4.9 fmol found in the cellular extract. Moreover, the data is close to Ewing's group by CE-LIF detection ( $2.9 \pm 1.1$  fmol),<sup>26</sup> which verified that this method was an effective way to determine DA within single cells. The data is higher than Cheng's group by CE-ECD with a single carbon fiber electrode ( $0.61 \pm 0.30$  fmol),<sup>22</sup> the reason might be due to the difference of differentiation of cells.

## Conclusions

In this paper, palladium nanoparticles modified carbon fiber microdisk electrodes were used as the working electrode of CE-ECD to detect DA at the first time. Compared with the single carbon fiber microelectrode which has been commonly used to determine the catecholamines, the fabrication of working electrode and the operation of individual cell analysis were simplified. At the same time, the Pd nanoparticles modified microdisk electrode reported in this paper provided an enhanced effective electrode surface and higher catalytic activity toward dopamine. Owing to the small sampling and fast separation capabilities of CE and high sensitivity of Pd nanoparticles modified electrode, the developed method is useful and powerful for chemical species analysis in single cells.

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