Original

Separation and determination of dopamine and epinephrine in serum by capillary electrophoresis with inkjet introduction system

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Abstract

A new inkjet sample introduction method was developed for capillary electrophoresis (CE). This system could control the exact volume of ejected liquid with high accuracy and precision at the picoliter level. The single droplet volume of 1×10^{-6} mol/L dopamine (DA) in 1×10^{-2} mol/L borate buffer and in the 40 times diluted serum were 240 pL and 243 pL, respectively. Sample droplets were ejected by stressing a piezoelectric slice stuck on the extrusion chamber. The pulse voltages were applied to the piezoelectric slice which made the slice deformed. Using this method, DA and epinephrine (EP) were separated and the concentration of EP in serum was determined at 5.88×10^{-7} mol/L, DA was not detected. The recovery ($n = 3$) of EP and DA added to serum were in the range of 95.8–98.7%, and 97.5–103.4% respectively, which showed good accuracy and reproducibility.

Keywords : Inkjet, Exact sample introduction for CE, Dopamine, Epinephrine

Introduction

Inkjet printer is one of the most commonly used printers. Inkjet printing essentially is a technology for dispensing precise amounts of liquid material with high positional resolution. The method is called drop–on–demand (DOD) [1,2]. Inkjet printing could be a promising method for droplet dispensing with high precision and reproducibility [3]. In these years, the inkjet technology has been used as a tool for rapid prototyping, small volume dispensing, and the production of small sensors [4–6]. The life science is also concomitantly progressing with new applications of DOD for precise dispensing of DNA and protein molecules [7–8].

CE was introduced in 1960s [9], which had rapidly evolved into the analysis of extensive range of targets, like DNA, peptides, protein and small ions. Therefore, it was used as the one of the popular separation technique, and made great contribution to environmental science and life science [10], like monitoring environmental condition, screening status in genetics. In accordance with the progress for material science, nanotechnology and molecular engineering, the separation efficiency of CE obtained significant progress [11–13]. However, comparing the separation potential of CE with other separation techniques, the quantitative capacity is not so compatible. It is due to the deficiency of proper sample introduction methods which couldn't supply exact introduction volume for CE.

DA and EP play the major roles as neurotransmitters in central and peripheral nervous systems [14]. DA is involved in the induction and in the expression of behavioral sensitization by repeated exposure to various drugs of abuse [15]. The neurobiological mechanism of drug abuse and addiction cannot be accommodated by a single, unitary hypothesis. Regarding to DA, only one among the many neurochemical systems are likely to be involved in drug abuse, a parsimonious hypothesis to provide only a partial account of its role in this condition. High catecholamine levels in blood are associated with stress [16]. Extremely high levels of catecholamine can occur in central nervous system trauma due to stimulation or

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damage of nuclei in the brainstem, which can be caused by neuroendocrine tumors in the adrenal medulla or caused by monoamine oxidase A deficiency. It also occurs the absence of pheochromocytoma, neuroendocrine tumor and carcinoid syndrome [17]. Nowadays, much interest has been focused not only on researching the bioactive function of the catecholamines but also on developing the selective methods for their detection in biological system [18–19]. DA and EP were normally detected by electrochemistry analysis. A novel taurine modified glassy electrode for determination of EP and DA, the linear range was 2.0×10^{-6} M to 6.0×10^{-4} M and 1.0×10^{-6} M to 8.0×10^{-4} M, respectively. And another group reported nano Pd–Au particles modified glassy carbon electrode for quantitative analysis of DA. The linear range was from 0.5 to 6.95×10^{-6} M [20–21].

In this study, the inkjet introduction system was applied to CE [22] and quantitative analysis of catecholamines in serum by accurate controlling the exact introduction volume was carried out. The inkjet microchip was used for precise placement and introduction of sample for CE. They say that there is only once or no false ejection out of one billion times ejections in inkjet technology. This issue also promised the consistency and reproducibility of ejection. For the accurate quantitative analysis, it is extremely important to know the exact amount of sample introduced into the capillary. The inkjet introduction method will be expected to solve the problem.

Experiment

Chemicals

All chemicals were of analytical grade except especially indicated and were used without further purification. Dopamine hydrochloride (DA) and the fluorescein isothiocyanate isomer I (FITC) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). L–Adrenaline Bitartrate (Epinephrine, EP) was from Tokyo Chemical Industry (Tokyo, Japan). Pyridine, Sodium tetraborate decahydrate, Sodium hydroxide, Hydrochloric acid, Acetone (for spectroscopy) were bought from Kanto Chemical Co., Inc. (Tokyo, Japan). The human serum used in the experiment was purchased from Bethyl Laboratories INC (TX, US), and containing 0.1% sodium azide. Deionized water (Milli–Q system, Japan Millipore Co., Tokyo, Japan) was used throughout the experiment.

Inkjet microchip

Four–channel inkjet microchip was kindly gifted from Fuji Electronics Co. (Tokyo, Japan) that was used as a printing head of industrial recorder (Fuji Electronics Co., Type CNO–1010, Tokyo, Japan). Sample droplets were yielded via the deformation of the extrusion chamber that was pushed by the piezoelectric slice driven by the pulse voltage. The number of ejected droplets could be controlled by the number of applied pulses between 1 and 10000. Furthermore, some parameters of pulse waveform could be adjusted as follows: applied voltage; 0～120 V, the pulse duration and interval; 1～100 μs.

Inkjet introduction system

The integrated equipment (including introduction, separation and detection device) has been described elsewhere [23]. The system is shown in Figure 1. Briefly, the inkjet microchip was moved by XY stage to align the center of capillary tip to the nozzle of inkjet microchip. In the injection process, the tip of capillary in the inlet reservoir was raised up and out of buffer solution. Then the inkjet microchip ejected definite amount of sample droplets. The sample on the capillary tip was pushed into the capillary by Laplace pressure caused by surface tension.

Separation and detection system

The setup for CE device was made by our laboratory. An in house built high voltage power supply (0–20 kV) was used to carry out CE separation. The length of capillary tube (O.D. 0.375 mm, I.D. 0.050 mm, GL Science, Japan) was 45 cm, and the effective separation length was 25 cm. A laboratory–made LIF detection system, containing a solid blue laser (HK–5517–02, 473 nm, 5.0 mW, Shimadzu, Kyoto, Japan), an incident light fluorescence microscope (BX40F4 Olympus, Tokyo, Japan) with a filter set (U–MWIB2 Olympus, Tokyo, Japan), a photomultiplier tube (PMT) (H6780–02, Hamamatsu Photonics, Shizuoka, Japan) and a laboratory–made amplifier. The chromatographic data–processing system (LAS5 Soft, Chiba, Japan) was applied to collect the fluorescence signal.

Labeling procedure

To match fluorescence detection, the derivatization of DA and EP with FITC was implemented according to the procedures described elsewhere [21]. All procedures were carried out in a 3 mL brown glass. 5×10^{-3} mol/L of DA and EP standard solutions were prepared with water. And 5×10^{-3} mol/L of FITC solution was prepared in acetone containing 1% pyridine. As the instability of FITC, the solution of FITC was freshly prepared before derivatization. And the DA and EP solutions were used within a week because both of them were easily decomposed. For the derivatization, 100 μL of above DA or EP solutions was mixed with 500 μL FITC solution and 400 μL 2×10^{-2} mol/L borate buffer solution (pH = 9.0), respectively. The mixed solution was kept at 25°C in dark for about 12 hours.

Preparation and derivatization of serum samples

The labeling process of the human serum was same as above. After the derivatization reaction was completed, the FITC–labeled

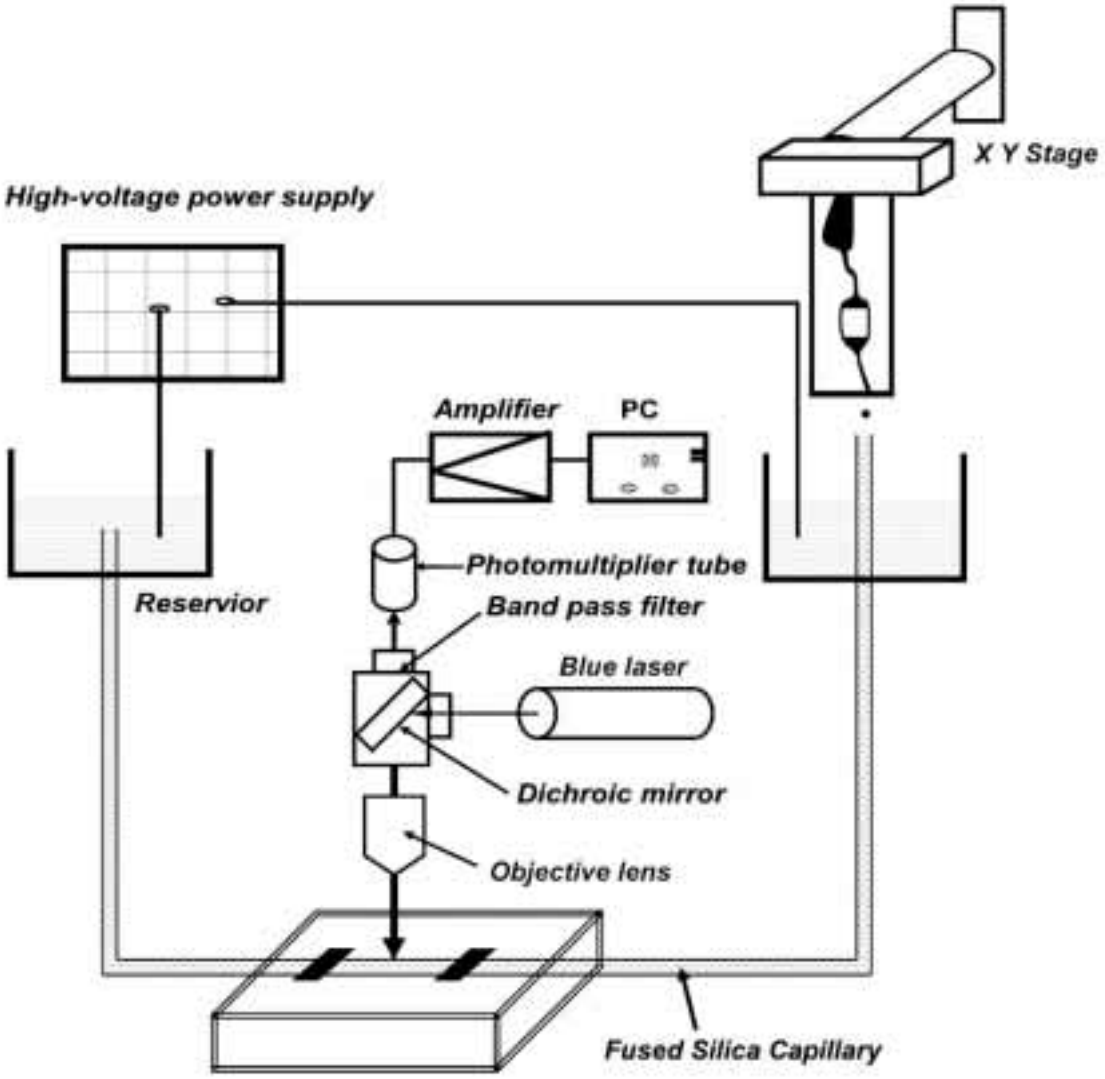

Figure 1. The diagram of inkjet introduction system with CE, LIF detector and the data processor.

serum was centrifuged at 1250 rpm for 5 min. The supernatant was collected and applied to CE separation. The FITC labeled serum was diluted for 4 times by 1×10^{-2} mol/L borate buffer solution (pH = 9.0) before ejection to CE. The final amount of acetone in diluted serum was 12.5%, which was also an important effect inkjet ejection and treatment of serum. All the sample solutions including FITC–DA, FITC–EP solutions and diluted FITC labeled serum was filtered through 0.45 μm PDVF (Kanto Chemical Co., Inc., Tokyo, Japan) membrane filter before injection to the CE system.

Accurate solution gravimetric

Accurate mass measurements of micro droplets measurements of micro droplets were carried out by the method as already reported by Verkouteren et. al. [24]. Briefly, micro balance with Sarto_In Timer (Sartorius mechatronics, Tokyo, Japan) was used to record the weight value. Compensation of evaporation was made from the time course variation of droplet weight.

Results and discussions

The principle of inkjet injection for CE

The process of liquid drop introduction in inkjet injection system was shown in Figure 2. According to the record of high speed camera (KEYENCE, VW–9000, Tokyo, Japan), all the droplets were ejected through inkjet as drop–by–drop. At first, the tip of the capillary was lifted from the buffer solution. Then driving pulses were applied to the piezoelectric slice. Consequently, the sample droplets were ejected from the nozzle of inkjet microchip and accumulated on the tip of capillary. Then, the accumulated droplets on the tip of capillary were pushed into capillary. Finally, the capillary was immersed back into the buffer reservoir to perform CE separation.

Surface tension is a property of the surface of liquid that allows

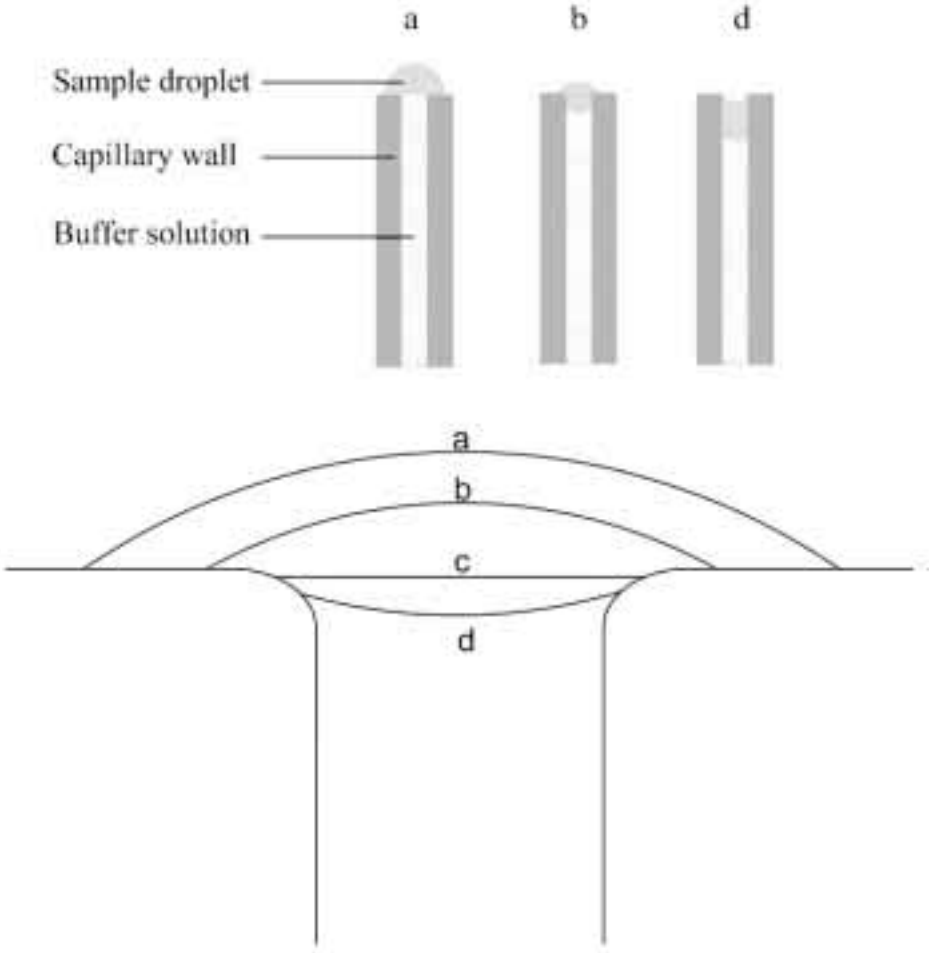

Figure 2. The process of liquid introduction of inkjet.

it to resist an external force, which is the important driving force to complete the liquid sample introduction in inkjet injection system for CE. This property is caused by cohesion of similar molecules, and is responsible for many behaviors of liquids. When the liquid surface is horizontal and flat, the external pressure p_{ex} equates the internal pressure p of the liquid. When the liquid surface is curved, the p_{ex} is different from p. If the surface is in the convexity, the surface tension γ in the vertical direction would generate a downward pressure as shown in Figure 2. The p_{ex} is greater than p until they are counterbalanced; oppositely if the surface is concave, the surface tension would cause an upward pressure in the vertical direction resulting in that $p_{ex} < p$. The difference of p and p_{ex} is defined as added pressure below curved surface Δp. In conclusion, on the convexity $\Delta p = (p - p_{ex}) < 0$, and on the concave $\Delta p = (p - p_{ex}) > 0$. And the direction of Δp (Laplace pressure) points to the center of the curved liquid surface.

According to Young–Laplace equation,

$$\Delta p = \frac{2\gamma\cos\theta}{r} \quad (\Delta p > 0) \tag{1}$$

$$\Delta p = \frac{2\gamma\cos\theta}{r_c} \quad (\Delta p < 0) \tag{2}$$

Where r surface curvature radius, r_c capillary internal radius, and θ contact angle.

As in the Figure 2 shown, the initial state 'a', the curvature radius was large. Then when the curvature radius of sample droplet was gradually decreased from 'a' to 'b'. The liquid drop was partly introduced into the capillary. The Laplace pressure is usually very large. For example, droplet with 10 μm of radius gives several thousand N/m^2 of surface tension. The direction of Laplace pressure at the stage 'a' is downward because the droplet surface is convex. Under the Laplace pressure, the sample droplet would be pushed into the capillary. In this stage, with the surface tension of droplet gradually pressing the liquid into the capillary, the surface curvature radius would be deceased; therefore the Laplace pressure was increased. The liquid drop was more rapidly pushed into the capillary. Finally, the liquid drop was completely pushed into the capillary, and the liquid surface went to 'c' as flat. On the condition 'c', the Laplace pressure was 0. Then, liquid surface was pulled to

form a concave surface like 'd' for the hydrophilic inner wall of capillary and the gravity of water pillar inside the capillary. Thus, the whole liquid introduction was completed.

The effect of inkjet ejection condition on the sample introduction reproducibility

The driving wave form for piezoelectric slice had two parameters, the driving pulse voltage and the duration of pulse. The speed of droplet was higher when the driving pulse voltage was higher. In the experiment, one electric driving pulse could eject one droplet from the inkjet. The droplets would not be ejected out, or deviate from normal track, when the driving pulse voltage was less than 23 V. The reason is the driving force was not enough to push out the droplets. In this case, the relative standard deviation (RSD) of sample introduction for CE was large, because it could not be guaranteed every droplet match the center of capillary on tip. On the other hand, the sample droplets would splash out from the tip of capillary when the voltage was beyond 50 V, which also caused the high RSD. All of the process of ejection was recorded by high speed camera. When the driving voltage was less than 50 V, every driving pulse could generate one droplet. Once the driving voltage was over 50 V, every pulse would generate one main droplet and a small satellite droplet. Since the satellites droplet was much smaller, it was easy to be effected by the current environment to generate high RSD. For example, the satellite droplet could be strayed away easily before touching the tip of capillary. And as shown in Figure 3, the optimum driving voltage of 25 V to 50 V for inkjet was obtained, while the RSD of sample amount introduced was less than 3%.

Evaluation the introduced volume

One droplet volume could be calculated by the quotient of the certain number of droplets mass and their density. So the volume of sample introduced could be figured out. Comparing other injection methods of CE, the inkjet introduction method gave absolute sample volume injected, which is of importance for the analytical method of standardization. As the results listed in Table 1, the droplet volume could be obtained by dividing the mass of 10000 drops sample with their density. Difference of the volume of single droplet was not obvious between DA solution and the diluted serum. While the inkjet driving conditions were selected as follows: driving pulse voltage 50 V, the duration of pulse drive 35 μs. Here,

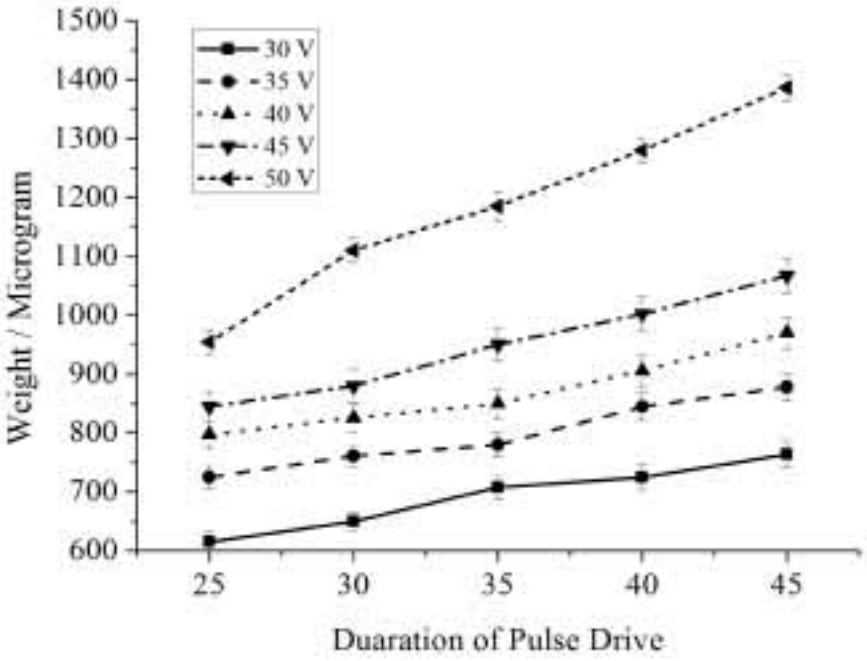

Figure 3. The effect of driving pulse voltage on the weight of 3000 droplets of water.

Table 1. The calculation of droplet volume.

	Weight 10000 drops (g)	Density (25°C) (g/mL)	1 droplet volume (pL)
DA solution (1.00×10^{-6} mol/L)	0.00241	1.004	240
40 times diluted Serum	0.00242	0.997	243

The injection conditions for inkjet: driving voltage was 50 V, drive pulse duration was 35 μs.

the single droplet volume of 1×10^{-6} mol/L FITC–DA in 1×10^{-2} mol/L borate buffer solution was 240 pL, and that of 40 times diluted serum (1×10^{-2} mol/L borate buffer solution) was 243 pL. In the experiment, the original derivative solution was diluted to various concentrations. In order to investigate the effect of different acetone amount (it was the main component of FITC labeled sample, which probably affect the surface tension of the liquid sample, even its droplet volume ejected form inkjet) on the droplet volume, the various concentrations of acetone solution were prepared from 0.001% to 20%, and the droplet volumes were obtained. As a result, 1 droplet volume was 246 ± 2.5 pL for the solution tested. Variation of 1 droplet volume showed no distinct difference among the varied concentrations of acetone solutions. Therefore, the affection of various concentrations of acetone on the sample droplet volume could be ignored.

Electrophoresis separation and method validation

DA and EP were labeled by FITC in advance. And FITC is a relatively large molecule comparing to DA and EP. FITC–DA and FITC–EP were separated on the baseline under the condition 1×10^{-2} mol/L borate buffer solution (pH = 9.0). As shown in Figure 4, FITC–DA and FITC–EP were separated within 350s. And we certificated the first peak was FITC, second one was FITC–EP, and the third was FITC–DA by adding individual standard solutions, respectively. The FITC appeared in 270s, EP stayed at 292s, and DA eluted at 310s.

As Table 2 shown, the linear dynamic relationships were observed between the fluorescent intensity and the concentration for both FITC–DA and FITC–EP. The calibration plots, obtained by CE of the standard solutions, showed the good linear relationship between the florescence intensity and concentration for FITC–EP and FITC–DA. The detection of limits were 1.78×10^{-9} mol/L for EP and 2.22×10^{-9} mol/L for DA. The regression coefficient con-

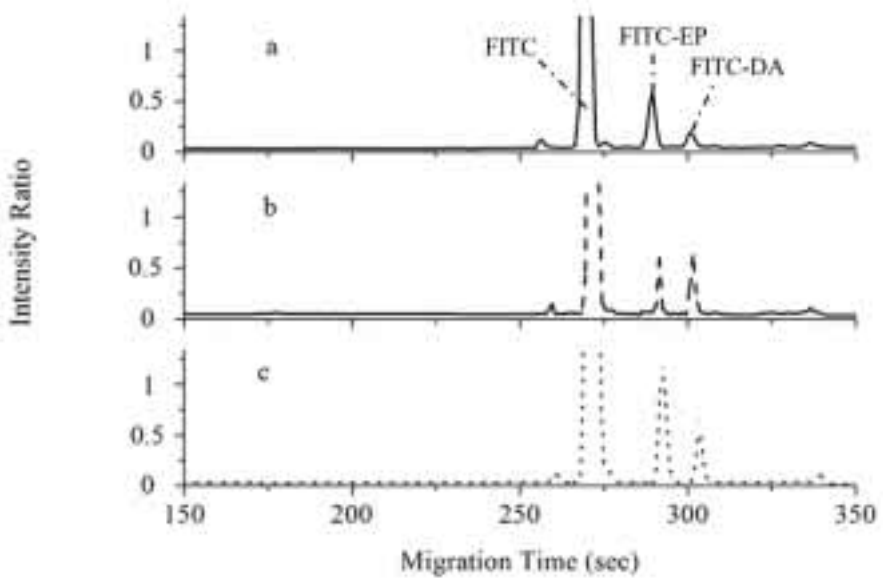

Figure 4. The separation electrophoretogram of FITC, FITC–DA and FITC–EP. (a) electropherogram of FITC, FITC–DA (5.00×10^{-7}mol/L) and FITC–EP (5.00×10^{-7}mol/L). (b) The electrophorogram after adding FITC–DA (the final concentration of FITC–DA was 1.00×10^{-6} mol/L). (c) The electrophorogram after adding FITC–EP and FITC–DA (The final concentration of FITC–DA was 1.00×10^{-6}mol/L, and FITC–EP was 1.00×10^{-6} mol/L). While the buffer was 1×10^{-2} mol/L borate buffer (pH = 9.0). The driving conditions for ink–jet: driving pulse voltage was 20 kV.

Table 2. Regression data, LOD and RSD for FITC–EP and FITC–DA.

Analyte	Regression equation	Regression constant	Linear range (mol/L)	LOD (mol/L)	RSD (%) (n=4)
FITC–EP	y = 3335 + 2203 x	0.998	$1.00 \times 10^{-8} - 2.00 \times 10^{-6}$	1.78×10^{-9}	1.12–2.75
FITC–DA	y = 2672 + 1665 x	0.999	$5.00 \times 10^{-8} - 1.00 \times 10^{-6}$	2.22×10^{-9}	1.64–2.89

y: fluorescence intensity (peak area)

x: analyte concentration

The injection conditions for inkjet: driving voltage was 50 V, drive pulse duration was 35 μs. The injected volume for FITC–EP and FITC–DA were 2.40 nL (10 droplets).

stants of DA and EP were 0.999 and 0.998 respectively.

The precisions were also listed in Table 2. The RSD of peak areas of FITC–EP and FITC–DA were in the range of 1.12–2.75%, and 1.64–2.89%, which demonstrated the good precision of the inkjet injection method for CE.

Quantitative determination of DA and EP in serum

The method was used for the analysis of EP and DA in human serum. A typical electropherogram is shown in Figure 5. The experimental results were summarized in Table 3. The EP content in the serum was determined around 5.88×10^{-7} mol/L, which was equal to 1.08 ng/mL. In the adult body, the concentration of EP is lower than 10 ng/mL [25]. And DA could not be detected. The recovery (n = 3) of FITC–EP was between 95.8–98.7%, and FITC–DA was between 97.5–103.4 %.

Conclusions

Sample introduction with inkjet was successfully applied to the determination of DA and EP in serum by CE. The exact sample introduction volume could be calculated accurately by this system. It is significant for analytical method standardization. In this experiment, the single droplet volume of 1×10^{-6} mol/L DA solution was 240 pL and 4 times diluted serum was 243 pL. As the inherent character of inkjet technique, precision of quantitative analysis was greatly improved [24].

Acknowledgments

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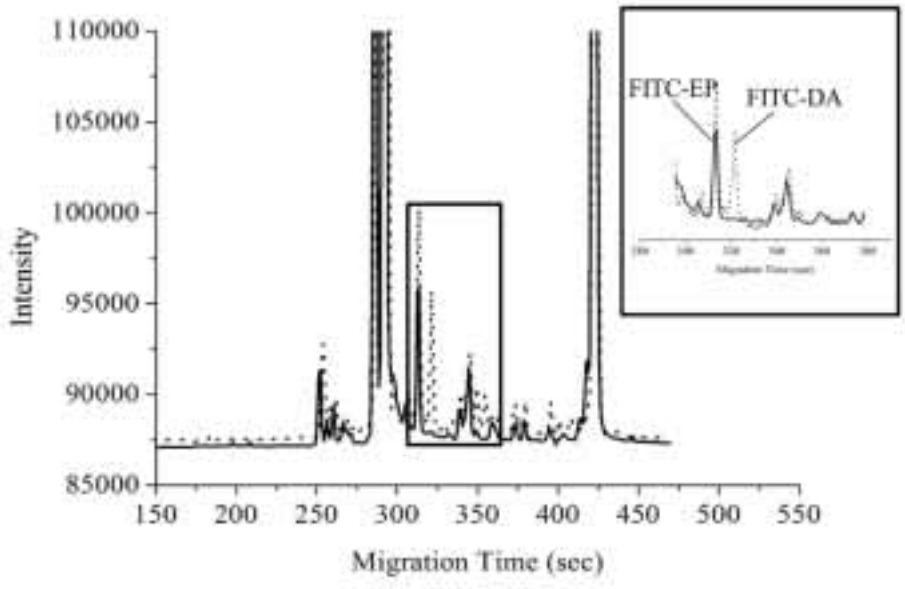

Figure 5. The electrophoretograms of serum sample. Buffer solution was 1×10^{-2} mol/L borate buffer, pH 9.0, applied pulse voltage for inkjet was 20 kV. The insert figure was the magnified view of FITC–DA and FITC–EP peaks. Solid line was the electropherotograms of serum sample. The dot line was the electrophoretogram added 1×10^{-7}mol/L of FITC–DA and FITC–EP.

Table 3. Analytical results and average of DA, EP in serum.

Sample number	Analyte	Amount added (mol/L)	Amount detected (mol/L)	Recovery (%)
1	EP	0	1.47×10^{-7}	None
	DA	0	NA	None
2	EP	1.00×10^{-7}	2.43×10^{-7}	95.8
	DA	1.00×10^{-7}	0.96×10^{-7}	97.5
3	EP	2.00×10^{-7}	2.94×10^{-7}	99.6
	DA	2.00×10^{-7}	2.07×10^{-7}	103.4
4	EP	4.00×10^{-7}	5.42×10^{-7}	98.7
	DA	4.00×10^{-7}	3.90×10^{-7}	97.5

The injection conditions for ink–jet were same to Table 2. The injected volume of 4 times diluted serum was 2.43 nL (10 droplets). NA means that did not detect.

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