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Electrophoretic separation of neurotransmitters on a polystyrene nano-sphere/polystyrene sulphonate coated poly(dimethylsiloxane) microchannel

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In this paper, a poly(dimethylsiloxane) microchip with amperometric detector was developed for the electrophoretic separation and determination of neurotransmitters. For increasing the separation efficiency, the microchannel is modified by polystyrene sulphonate/polystyrene nano-sphere self-assembly coating. A stable electro-osmotic flow (EOF) and higher separation efficiency are obtained in proposed modified microchannel. Under optimized conditions, dopamine, epinephrine, catechol, and serotonin are acceptably baseline separated in this 3.5 cm length separation channel with the theoretical plate number from 4.6×10^4 to 2.1×10^5 per meter and resolution from 1.29 to 12.5. The practicability of proposed microchip is validated by the recovery test with cerebrospinal fluid as real sample which resulted from 91.7% to 106.5%. © *2011 American Institute of Physics*. [doi:10.1063/1.3609968]

I. INTRODUCTION

Considerable interests have been focused on the lab-on-a-chip technique over last decade. Microchips have offered remarkable advantages including high throughput, high efficiency, reduced cost, and minimized consumption of sample and reagents.[1–5] Poly(dimethylsiloxane) (PDMS), an attractive polymeric material, was introduced to fabricate the microchip first by Effenhauser for the separation of DNA fragments in 1997.[6] Thence, PDMS has become the most popular microchip material mainly due to its advantages of easily handling, good seal properties, and optical transparency.[7–10] However, there are also some limitations. The electro-osmotic flow (EOF) on PDMS microchannel is usually unstable and poorly controlled because of its complex surface property. Some organic solvents or hydrophobic analytes are inclined to be adsorbed onto its hydrophobic surface. Series of chemical or physical methods including graft polymerization, plasma treatment, UV/ozone treatment, silanization, adsorption of detergents, proteins, and polyelectrolytes have been used to modify the PDMS microchannel to meet the requirements in various applications.[11–17] For example, Wang *et al.*[12] have layer-by-layer modified the PDMS microchannel with a straight-chain polymer (polyethyleneimine) and gold nanoparticles; Sui *et al.*[13] performed an *in situ* polyethylene glyco (PEG) modified surface on pre-oxidized PDMS microchannel and then incubated with 2-[methoxy(polyethylenoxy)propyl] trimethoxysilane for 2–3 days to obtained a more hydrophilic PDMS surface with 40° of contact angle.

Polystyrene (PS) microsphere is widely used as the modifier for chromatographic column. Recently, some researchers attempt to utilize it in capillary electrophoresis and microchips.[18–25] Bakry *et al.*[20] have reported that a fused silica capillary with PS latex particles modified on the inner wall could be used for the purification and enrichment of phosphor-peptides or His-tagged proteins. Yang *et al.*[21–24] described the modification of a PDMS microchannel by PS colloidal

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particles, which was used as an *in-situ* optofluidic microsensors or light filters with the built-in colloidal photonic crystals. Harrison *et al.*[25] first reported a microfluidic system for the sized separation of biomolecules within self-assembled three-dimensional ordered PS colloidal lattices. However, to the best of our knowledge, there is no report about electrophoretic separation on self-assembled nano-PS modified PDMS microchannel.

Poly(styrenesulfonate sodium salt) (PSS), a polyanion, could be firmly adsorbed on the surface of positive charged polymer. Xiao *et al.*[26] used alternating layers of poly(dimethylammoniumchloride) (PDDA) and PSS to form a $(PDDA/PSS)_2$ modified PDMS microchannel, which achieved 100 multiples improvement of separation efficiency. Qiu *et al.*[27] also reported a similar $(PDDA/PSS)_2$ modified PDMS microchannel to obtain a stable EOF due to reduced non-specific adsorption.

In this study, the positive charged PS nano-sphere (PSNS) has been solvothermal synthesized[28] and was self-assembly modified[29] onto a PDMS microchannel to obtain a quasi-ordered PSNS monolayer. Followed by driving through the PSS solution, the final PSNS/PSS modified layer was built on channel surface and the surface properties were characterized by EOF, contact angle. Dopamine (DA), epinephrine (EP), serotonin (5-HT), and catechol (CA) were separated with remarkable higher resolution on the modified microchannel than that on the native one. The method was applied to detect the analytes in cerebrospinal fluids (CSFs) to validate its practicability.

II. MATERIALS AND METHODS

A. Instruments and reagents

The voltage for electrophoresis is supplied by a custom-built power-source (Nanjing Hengyue Electro-optical Technique Co., China). It has a voltage ranging from 0 to 5000 V. Electrochemical detection is performed using a RST3100 Electrochemical Workstation (Suzhou Reisite Instruments Co., China). A CVM-100E Stereo Microscope (Shanghai Changfang Optical instrument Co., Shanghai, China) is applied for surveys in experiments and also to record the surface morphology. A JC2000C1 optical contact angle analyzer (Shanghai Zhongchen Digital Technique Instruments Co., China) is used for contact angle measurement. The size of PSNS is determined by a HPP 5001 Laser Particle Size Analyzer (Marvern, England).

Sylgard 184 (PDMS) silicone elastomer and curing agent are obtained from Dow Corning (Midland, MI, USA). DA, EP, 5-HT, and CA are purchased from Sigma-Aldrich. Na_2HPO_4, KH_2PO_4, acetic acid, sodium aceticum, styrene, acetone, and PSS are obtained from Nanjing Chemical Reagents Factory (China). All other reagents are of analytical grade. All solutions are prepared with doubly distilled water and passed through a 0.22 μm cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China).

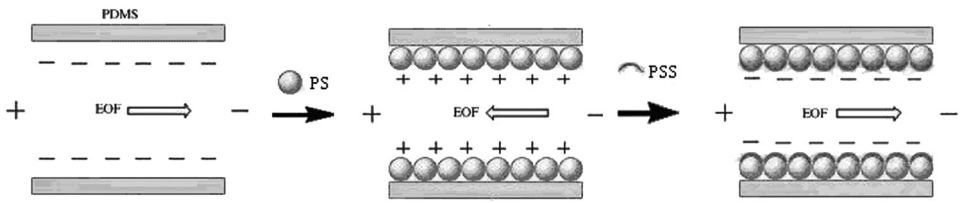
B. Microchip

1. Fabrication of PDMS microchip

The PDMS chips with cross-channel are fabricated as previously described.[12] A micro-photolithographic fabricated GaAs mold was used for casting the chips. The separation channel is of 3.90 cm (3.50 cm of effective length) with 50 μm of width and 18 μm of depth. A 1.0 cm sampling channel crosses the separation channel at 0.4 cm from one end with 30 and 18 μm of width and depth, respectively. A degassed 10:1 mixture of precursor and curing agent of Sylgard 184 was poured onto the GaAs mold, and then cured for 2 h at 70 °C. After the cast replica was peeled from the mold, 3 mm diameter holes were punched as the sampling and buffer pool at both ends of the channels. A 3 mm thick PDMS plate was cast by a glass mold in the same procedure. Both two sheets were ultrasonically cleaned subsequently with acetone, ethanol, and water, finally dried under infrared lamp, and the PDMS microchip was self sealed.

2. Assembling of PSNS/PSS layer

Positively charged polystyrene nano-spheres are synthesized by solvothermal method. A 6 ml styrene monomer, 75 ml acetone, 75 ml doubly distilled water, and 0.07 g V-50 (2,2-azobis-



SCHEME 1. Schematic representation of the channel wall coating. The length of the channel is 3.50 cm.

(isobutyramidine)dihydrochloride) initiator of polymerization were blended together in an autoclave, stirred for 10 min, and then cured for 6 h at 90 °C.[28] The size of the PSNS was determined for approximately 65 nm of the most probable diameter with a 20 nm half-peak width of distribution.

The stage of modification could be seen in Scheme 1. The ordered monolayer of PSNS was obtained by self-assembly at air/water interface. PSNS colloidal suspension of 100 μl was diluted with acetone in equal volume. The suspension was dispersed onto the surface of ultrapure water in a ϕ12 cm Petri disk to form an ordered monolayer spontaneously. The cleaned PDMS sheets were then placed into the Petri disk tilted at 30°–40°. The ordered monolayer of the PSNS was therefore transferred onto them by careful drainage of water at a controlled rate.[29] After dried at room temperature overnight to remove the remaining solvent, the PSNS outside the channels were wiped off. Then, the PSNS-coated microchannel was built up by sealed together of two PDMS sheets. Finally, the PSNS-coated microchannel was further modified by pumping through the PSS solution (0.25 wt. % in water) for 1 h.

C. Electrophoresis

The electrophoresis arrangement is same as previous report.[30] The microchip was mounted on a plexiglass holder, which integrated a three-dimensional fine-tuning system with the precision of 1 μm in each direction. The amperometric detector was located in the detection reservoir (at the outlet end of channel) and consisted of an Ag/AgCl wire reference electrode, a Pt wire counter electrode, and a lab-made end-column carbon disk working electrode (I.D. 30 μm). Electrochemical detection was performed using chronoamperometry. All experiments were performed at room temperature.

D. Sample preparation

The CSF samples were obtained from volunteer patients with cerebral infarction (sample A) and intracranial infection (sample B). The samples were firstly centrifuged at 12 000 rpm for 15 min and the supernatants were collected. To precipitate the proteins, the isometric acetonitrile was added into it. Then, the supernatants were collected for assay by centrifugation at 12 000 rpm for 15 min again.

III. RESULTS AND DISCUSSION

A. Surface morphology of PSNS modified PDMS channel

Fig. 1(a) shows the scanning electron microscope (SEM) image of PSNS. It could be seen that PSNS was well-dispersed with smooth surface. It is shown that PSNS was assembled as a quasi-ordered and close-packed monolayer on the PDMS surface after the solvent was completely evaporated (Fig. 1(b)). This close-packed structure ensured that PDMS channel was coated by the homogenic wall of PSNS.

The PSNS monolayer was firmly held on the surface of the PDMS channel. It is due to the elasticity of PDMS that provided the effects as keystone, clamp and/or anchor, and hydrophobic interaction between PSNS and PDMS.[31,32] After that, PSS solution was pumping through that coated channel for 1 h. According to previous report,[33] negatively charged PSS could be adsorbed on those positively charged PSNS.

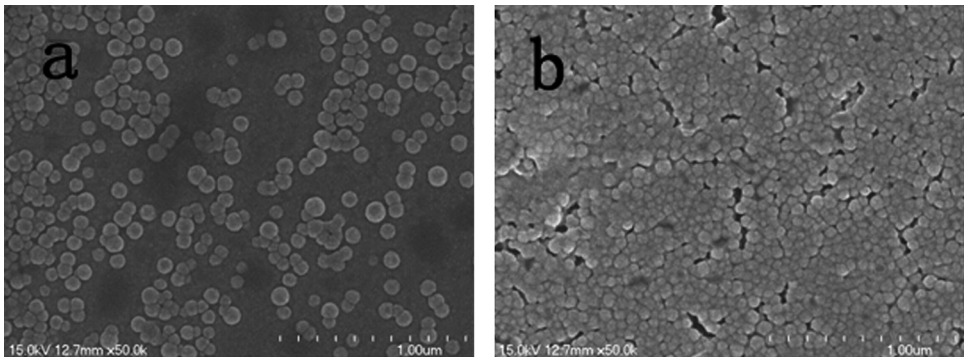


FIG. 1. The SEM image of (a) monodispersed PSNS and (b) PSNS array on PDMS.

B. The EOF in coated channel

The EOF is a very important influential factor for electrophoretic separation efficiency. It was monitored by the current measurement[34–36] and calculated according to $\mu_{EOF} = L^2/Vt$, here the L is the length of separation channel, V is the voltage of separation, and the t is the time of buffer arriving. It is commonly influenced by the *p*H of buffer solution. In 20 mM PBS solution, the influence of *p*H on EOF in PSNS/PSS coated channel was investigated within the *p*H range from 4.0 to 10.0 (Fig. 2). Compared with the native PDMS microchannel, the EOF is lower but more stable in testing *p*H range in the coated microchannel. The decreased EOF is favorable for the separation of neurotransmitters because they had similar migration times in the short channel. The lower and stable EOF in PSNS/PSS coated microchip might be attributed to the surface structure and electrostatic situation of the channel surface. As can be seen in Fig. 2, the EOF obviously decreased with the increasing *p*H of buffer (curve a). Opposite with the generally reported fact that the EOF would increase with the increasing buffer *p*H,[12] it seems to be an abnormal incident. But it is not a sole case in electrophoresis. Chen *et al*. have also reported the similar phenomenon in their research.[37] In complicated factors on EOF,[38,39] the surface adsorption of different charged species on PDMS might be the principal cause to induce

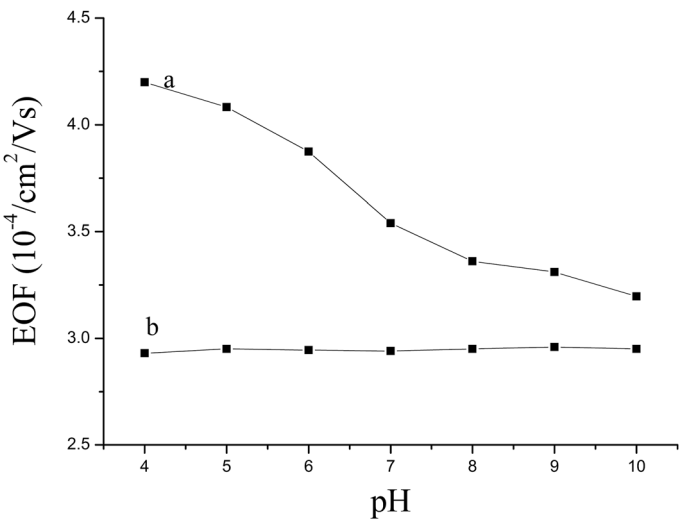


FIG. 2. The effect of buffer *p*H on EOF in (a) native PDMS microchannel and (b) PSNS/PSS coated PDMS microchannel under 1000 V of separation voltage with 20 mM PBS as running buffers.

TABLE I. The comparison of separation parameters of present work with reported works.

Method	n_{Theory} (number/meter)	EOF 10^{-4} (cm^2/Vs)	$R_{DA/EP}$	$R_{DA/CA}$	$R_{DA/5\text{-}HT}$	Ref.
Native PDMS microchannel	DA 9.0×10^3 EP 9.0×10^3 CA 4.0×10^4 5-HT 6.0×10^4	3.57	0	3.57	0.54	Present work
PSNS-PSS modified PDMS microchannel	DA 7.5×10^4 EP 4.9×10^4 CA 4.6×10^4 5-HT 2.1×10^5	3.06	1.29	4.41	12.5	Present work
PDDA-silica nanoparticle	DA 1.40×10^5 EP 1.39×10^5	2.21	1.7	…	…	Ref. 30
PDDA-gold nanoparticle	DA 9.7×10^4 EP 9.5×10^4	1.14	1.5	…	…	Ref. 40
PDDA-Gox	DA 2.19×10^5 EP 1.76×10^5 5-HT 1.89×10^5	0.75	1.84	…	1.03	Ref. 41
glass microchip	DA 5.61×10^3 EP 9.78×10^3 CA 1.87×10^4	…	1.36	1.81	…	Ref. 42
CE	DA 7.1×10^4 EP 1.0×10^4 5-HT 1.9×10^4	…	3.36	…	6.4	Ref. 43
HPLC	DA 1.4×10^4 5-HT 2.2×10^4	…	…	…	3.46	Ref. 44

the incident in a range of *p*H. Fortunately, after modification of PSNS/PSS on PDMS channel, a normal performance of EOF upon the *p*H appeared (curve b). Due to there are abundant surface groups of amino on PSNS and PSS being a strong electrolyte, in this case, the surface of coated channel is covered by the densely packed negative charges of PSS which were integrated on PSNS by the electrostatic action. So, there the lower EOF is resulted from the quasi-ordered rough morphology of modified channel surface with PSNS, and the better stability of EOF can be attributed to the buffering of surface charging from the PSS within the *p*H range from 4.0 to 10.0. It counteracted the influence of buffer *p*H to obtain a reproducible separation performance. On other hand, in this modified channel, the EOF is greater than those reported method (see Table I). Although it means that the elution in this channel was faster than those works, it is otherwise propitious to reduce the longitudinal diffusion to get higher separation efficiency.

C. Hydrophilicity of coated channel

It is one of the most important goals of modification of PSNS/PSS to improve the hydrophilicity of PDMS microchannel. The measurement demonstrated it with decreased contact angle from 109° to 76° after modification (see Figs. 3(a1) and 3(b1)). It could also be more intuitively observed from the images of water droplet on the channels (see Figs. 3(a2) and 3(b2)). The droplet on the modified microchannel spreads along the channel and got a spindle-shape while that of circular shape on native PDMS microchannel. The better soakage of channel surface is also profitable for decreasing the surface tension to allow a fluent and stable EOF.

D. Electrophoretic separation efficiency on the coated channel

An acetate solution of *p*H 6.0 was adopted as running buffer for the separation of three neurotransmitters (dopamine, epinephrine, and serotonin) and catechol. Fig. 4 gave the electrophorograms of the analytes in native channel (Fig. 4(a)) and coated one (Fig. 4(c)). Resulted from lower EOF in modified channel, the migration times of analytes were all prolonged, meanwhile the separation efficiency between four analytes were greatly improved. As illustrates in

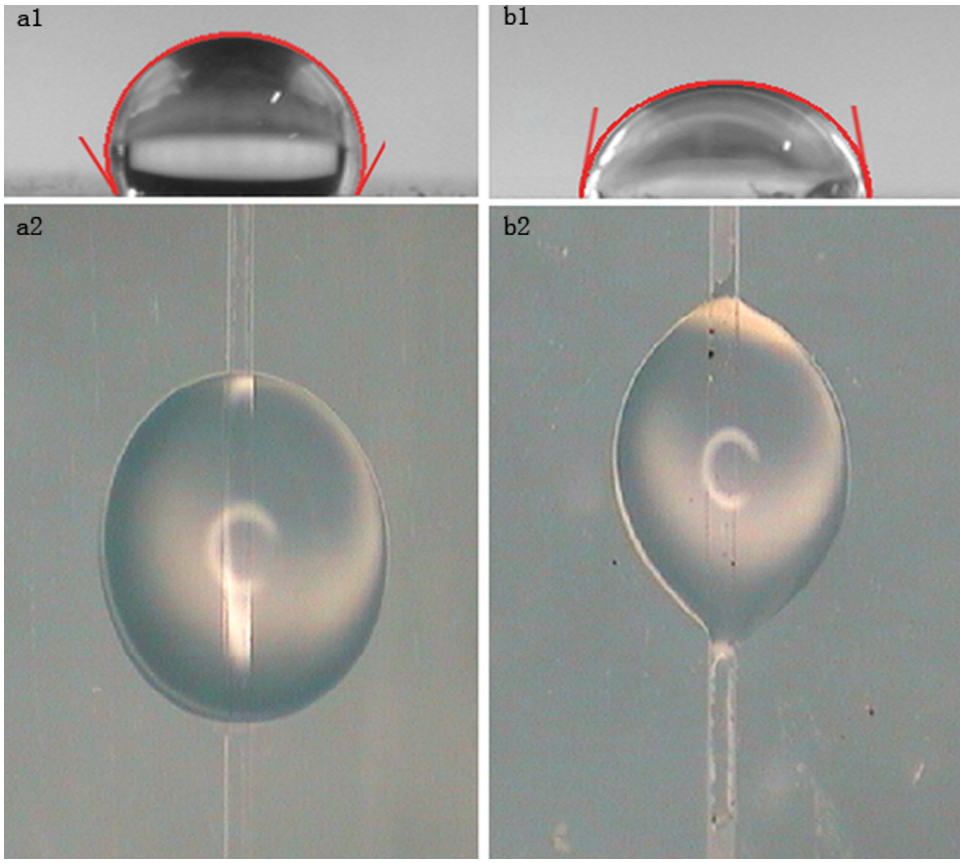


FIG. 3. The water contact angle on (a1) native PDMS and (b1) PSNS/PSS coated PDMS and corresponding droplet images (a2), (b2).

Fig. 4(a), the separation of 5-HT, DA, EP, and CA were very poor on native PDMS microchip. The resolution (R) of DA with 5-HT is only 0.37 and EP is not separated from DA at all. On PSNS/PSS modified PDMS microchip, 5-HT, DA, EP, and CA are well separated. The plate numbers for EP, DA, CA, and 5-HT at 1000 V of separation voltage are calculated as

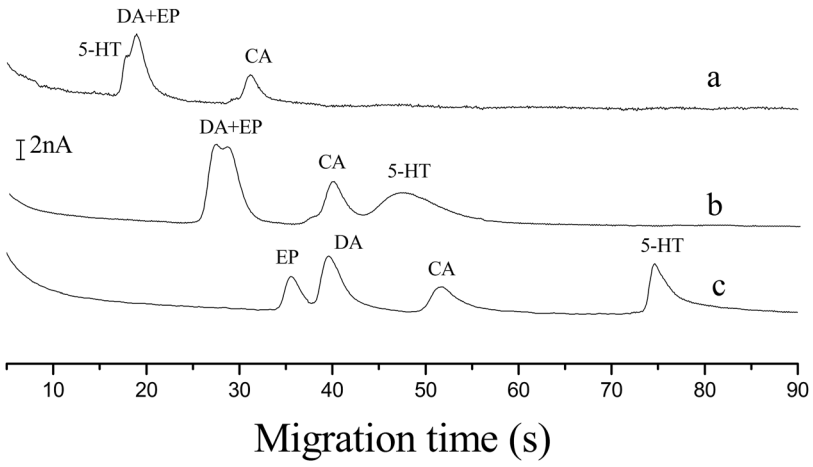


FIG. 4. Electropherograms of 50 μM EP, DA, 5-HT, and 40 μM CA on (a) native PDMS microchip, (b) PDDA/PSS coated PDMS microchip and (c) PSNS/PSS coated PDMS microchip. Sampled at 200 V for 1 s and detected at 0.60 V vs. Ag/AgCl.

TABLE II. Reproducibilities of PDMS chips coated with PSNS/PSS (n = 5).

	Average EOF 10^{-4}cm^2/Vs	RSD%
Run-to-Run	3.10	0.52
Chip-to-Chip	3.06	2.91
Day-to-day	3.12	2.68

7.5×10^4, 4.9×10^4, 4.6×10^4, and 2.1×10^5 per meter. Here the smallest R reaches to 1.29 which fulfilled between DA and EP, means an acceptable baseline separation. Compared with some previous reported works[30,40–44] (see Table I), the present work provided accepted even better results. It suggests that the approach to modify the microchannel with PSNS/PSS in this work was successful to improve the separation efficiency.

A bilayer of $(PDDA\text{-}PSS)_2$ modified PDMS microchip[26] is always reported as an efficient measure for improvement of capillary separation. The separation of the four analysts was also carried out in a $(PDDA\text{-}PSS)_2$ modified PDMS microchip (Fig. 4(b)) for comparison. It was found that the separation was a little improved than the native PDMS microchip; howbeit, the EP and DA could not be separated at all. By the results of obviously different separation efficiencies in two cases in despite of both PSS presented, it could be concluded that the presence of PSNS in modified layer was a critical role for better separation of those neurotransmitters and catechol.

According to the equation[45]

$$R = \left(\frac{1}{4\sqrt{2}}\right)(\Delta\mu_{app})\left(\frac{V}{D(\mu_{eo} + \bar{\mu})}\right)^{1/2}.$$

The resolution (R) of the separation in electrophoresis relates to electroosmotic mobility (μ_{eo}) and the average electrophoretic mobility of the analytes ($\bar{\mu}$). Better separation would be obtained at slower elution mobility. Compared with PDDA polymer, the quasi-ordered nanostructure of PSNS monolayer on inner-wall of the microchannel caused slower elution mobility.[46] Moreover, although the PSNS was covered by PSS, there might be some uncovered phenyls on their surface. They provided the opportunity for π-π interaction between themselves and the analytes.[47–49] This interaction not only enhances the retention of the analytes but also modulates the selectivity.

E. Stability of coated layer and linear detection range on the microchip

The reproducibility of PSNS/PSS modified PDMS microchannel is evaluated with relative standard deviation (RSD, n = 5) of EOF. The results are shown in Table II. The run-to-run RSD is 0.52% and the day-to-day RSD is 2.68%, showing good enough reproducibility. Furthermore, the chip-to-chip reproducibility is also good (RSD = 2.91%, n = 5). Under optimal conditions, the linear detection ranges and the detection limits (S/N = 3) of four analytes are listed in Table III.

TABLE III. The linearly ranges and detection limits for analytes.

Analyte	Linear range(μM)	Detection limit(μM)	Calibration equation I/nA (C/μM)	Correlation coefficient (r)
DA	10–600	2	$I = 2.16 \times 10^{-2} + 1.32 \times 10^{-2}$ C	0.9983
EP	10–500	2	$I = 2.3 \times 10^{-2} + 5.2 \times 10^{-3}$ C	0.9964
5-HT	10–600	6	$I = 1.49 \times 10^{-2} + 8.05 \times 10^{-3}$ C	0.9911
CA	5–400	1.5	$I = 2.61 \times 10^{-2} + 2.76 \times 10^{-3}$ C	0.9979

TABLE IV. The detection results and recoveries.

Compound	Sample	Detected concentration μM	RSD % n = 3	Recovery %	RSD % n = 3
DA	A	ND	ND	94.6	3.9
	B	ND	ND	98.6	1.8
EP	A	ND	ND	106.5	4.2
	B	ND	ND	100.4	2.6
5-HT	A	ND	ND	91.7	2.7
	B	ND	ND	94.5	3.2
CA[a]	A	4.0	2.6	104.3	3.7
	B	2.7	4.8	101.9	4.1

[a]The data have been conversed to the contents in original samples.

F. Application

Samples A and B are obtained from patients with cerebral infarction and intracranial infection. The pretreated samples were adopted as the practical models to validate the applicability of proposed modified microchip. Under above mentioned conditions, the determined results and recoveries of four analytes in CSF are listed in Table IV, indicate the good accuracy of proposed methods.

IV. CONCLUSION

A novel method to modify the PDMS microchip with PSNS/PSS was described for efficient separation of neurotransmitters. The characterization of the modified PDMS microchip was systemically investigated by SEM, EOF, contact angle, etc. Lower and stable EOF was obtained in proposed PSNS/PSS modified PDMS microchannel. On this basis, DA, EP, CA, and 5-HT were well separated with adequate separation efficiency.

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