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Dynamic coating for resolving rhodamine B adsorption to poly(dimethylsiloxane)/glass hybrid chip with laser-induced fluorescence detection

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Abstract

In this paper a method was described about dynamic coating for resolving rhodamine B (RB) adsorption on a hybrid poly(dimethylsiloxane) (PDMS)/glass chip. The results showed that when the non-ionic surfactant Triton X-100 was higher than 0.5% (v/v) into the phosphate buffer, the adsorption of RB appeared. Besides, some separation conditions for RB were investigated, including concentration of Triton X-100, concentration and pH value of running buffer, separation voltage and detection site. Through comparing electroosmotic flow, plate numbers and other parameters, an acceptable separation condition was obtained. Under optimized conditions, the precisions of RB detection (R.S.D., $n = 10$) were 2.62% for migration time, 4.78% for peak height respectively. Additionally, RB concentration linearity response was excellent with 0.9996 of correlation coefficient between 1 and 100 μM, and a limit of detection (S/N = 3) was 0.2 μM. Finally, we separated rhodamine B isothiocyanate and lysine deriving from the fluorescent probe, and the result displayed that the dynamic coating method was applicable by CE separations using PDMS/glass chip.

Keywords: Dynamic coating; Rhodamine B adsorption; Poly(dimethylsiloxane); Triton X-100

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1. Introduction

In recent years, micrototal analysis systems (μ-TAS) have been widely applied in a variety of fields and have been innovated for chemical and biological analysis [1–5]. To meet the requirements of easy fabrication, low cost, and great versatility, polymer-based microfluidic devices are rapidly gaining popularity and many substrates have been successfully used for μ-TAS, such as poly(methylmethacrylate) (PMMA), polycarbonate (PC), polyvinylchloride (PVC), polystyrene, polyurethane, and poly(dimethylsiloxane) (PDMS) [6–13].

As a chip material, PDMS is attractive and popular for it has some properties, such as ease of fabrication, low cost, nontoxicity, relatively low curing temperature, and ease of sealing with glass and other materials [9]. Moreover it is elastic and this makes it possible to be peeled from micromolds without damaging the micro-channels or master. So this kind of chip can be reused through ultrasonic rinsing with ethanol and water when clogged. In addition, PDMS chip has good optical properties that greatly facilitate optical detection, such as laser-induced fluorescence (LIF) [14].

Despite these advantages, PDMS has a lot of drawbacks to be overcome before it is considered as a material for electrophoresis microsystems [11,15,16]. PDMS is a bulk polymer consisting of repeated units of $-OSi(CH_3)_2O-$. The surface is hydrophobic and can adsorb nonpolar hydrophobic analytes due to its cross-linked hydrophobic chain with methyl branch. So it is difficult to wet with aqueous solvents, and owing to little negative charge on the surface of PDMS

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channel, electroosmotic flow (EOF) is much less than in glass or quartz ones [17–19].

Functional groups on material surface are important for microfluidic devices. Some modifications are in widespread use to alter the surface properties. In the field of μ-TAS some goals are very typical, e.g., to decrease the biomolecular adsorption, to alter the lubricity of the surface, to change the attachment of a biologically active molecule, or to increase the hydrophilic or hydrophobic character of the surface. In order to alter the hydrophobicity of PDMS surfaces, various chemical modifications have been performed. Wet chemical oxidation achieves hydrophilicity but gives a rough surface. Oxidation is often performed by exposing PDMS to various energy sources, such as oxygen plasmas [20,21], corona discharge [18,22] and ultraviolet light [23], to oxidize the polymer surface, and creating an inorganic silica-like phase. However, while these treatments greatly increase the wettability of the PDMS surface, the effect of the oxygen treatments is short-lived [11,13,17,24,25]. Recently, some other methods have been applied to deal with the adsorption of dyes on polymeric substrates [17,18,26–29]. Dynamic coating with polyelectrolytes provides a suitable approach [24,26,30]. Adopting sieving agent in solution, e.g., gel electrophoresis fulfills the separation of small molecules and DNA fragments [27].

In this experiment, a hybrid microfluidic chip of PDMS/glass was used, and LIF was adopted as detection method. It is known that LIF detection is only applied into the analytes with a fluorescence band or tagged with a fluorescent group. However the analytes and the derivatizing reagents are readily to be adsorbed onto PDMS surface due to their hydrophobic groups, that makes them separation and detection difficult. To resolve this problem, we successfully employed nonionic surfactant of Triton X-100 as additive for reducing RB adsorption. It is known that each surfactant has a critical micelle concentration and they include a long carbon-chain (C-chain). When added to the buffer at an enough high concentration, the strongly hydrophobic C-chain will interact with the PDMS channels and the adsorption of analytes will be controlled.

2. Experimental

2.1. Materials and chemicals

RB was obtained from the British Drug Houses LTD. (Poole, UK). Triton X-100 and rhodamine B isothiocyanate (RBITC) were purchased from Sigma Aldrich Co. (St. Louis MO, USA). Sylgard 184 was obtained from Dow Corning (Midland, MI, USA). Lysine (Lys) was obtained from Shanghai Chemical Reagent Co. (Shanghai, China). All reagents were of analytical-reagent grade and used without further purification.

The solutions for electrophoresis procedure were prepared with Milli-Q water. Background electrolyte solutions were prepared from stock solutions of 100 mM and stored at 4 °C. The surfactant of Triton X-100 was prepared freshly, and the stock concentration was 10% (v/v). Sample solution was prepared by diluting 2 mM RB stock solution in the corresponding electrophoresis buffer.

2.2. Apparatus

All experiments were performed on the Micralyne Microfluidic Tool Kit (μTK) (Micralyne Inc., Edmonton, Alta., Canada), which mainly consisted of high-voltage (HV) power supplies and a LIF detection system. The voltage outputs could be chosen with different modes among ground, floating and HV state. The instrument was equipped with 532 nm ν-doubled Nd–YAG laser (4 mW). The luminescent confocal detection module employs a 40 × 0.55 NA aspherical lens to focus the beam onto a channel of the chip. The optical path into PMT has a 10 nm bandpass filter centered at 568.2 nm. The optics diagram of the μTK was shown in the paper by Crabtree et al. [31]. Control and data collection was performed with a LabView (National Instruments, Austin, TX) software. In the experiments the acquisition frequency of the PMT was 25 Hz and the gain was 0.300.

2.3. Fabrication of PDMS/glass chip

Microfluidic channel patterns and the corresponding master were designed and fabricated as described previously in our group [32,33].

In brief, PDMS microchannels were got from molding on lithographically and wet chemically prepared glass master. The PDMS replica with cross-electrophoresis channel had the feature of 32 μm wide and 20 μm deep. After the PDMS replica was created with four reservoirs at each end of the cross-channel using a hole punch, a hybrid chip was created through sealing of the PDMS slab to a flat glass. The sketch was shown in Fig. 1.

2.4. Electrophoresis procedure on PDMS/glass chip

Prior to electrophoresis, the channels of the hybrid chip were flushed with Milli-Q water, 0.1 M NaOH and Milli-Q water, respectively, for 10 min, then with the separation buffer for 15–20 min. These solutions were directly introduced into the reservoirs, flushed through channels under vacuum. Before a separation was carried out, the buffer in the sample reservoir (SR) was replaced by the sample, and the four pins were inserted in the corresponding reservoirs (see Fig. 1). The sample injection was performed by two steps, that the first step is applying 300 V to pin 3 and ground to pin 4 (pin 3–pin 4) for 15 s, the second step is applying high voltage to pin 3 and ground to pin 1 (pin 3–pin 1). Once the injection is completed, the pinched separation is carried out, that the high voltage is applied to buffer reservoir (pin 2), low voltage to sample reservoir and sample waste reservoir (pin 3/pin 4), ground to buffer waster reservoir (pin 1) (see Fig. 1C and D).

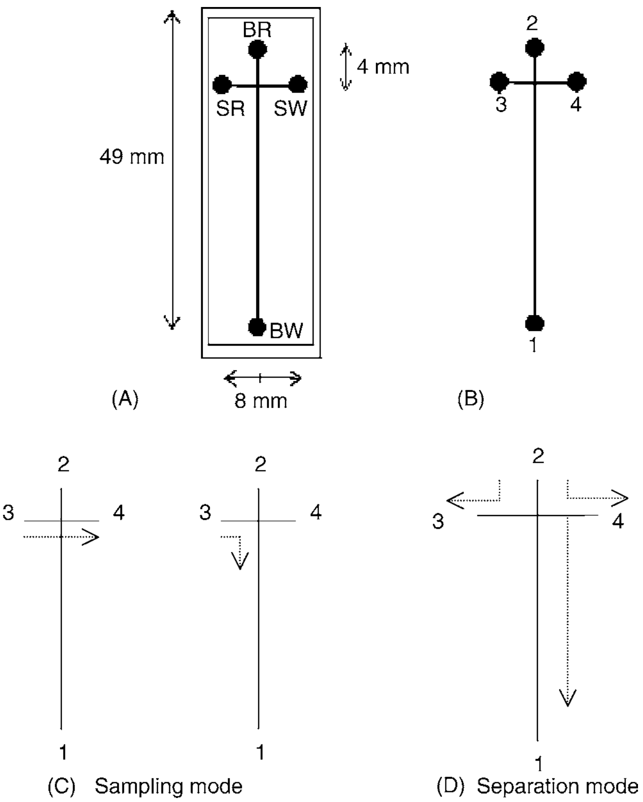

Fig. 1. Schematic of microchip layout (A, B), and schematic of sampling mode and separation mode (C, D). (A) All distances from cross-connection point to sample reservoir (SR), buffer reservoir (BR), sample waste reservoir (SW) are 4 mm. The length of effective separation channel is 45 mm. And the channels are 32 μm wide and 20 μm deep. (B) 1, 2, 3 and 4 correspond to the pin socket numbers on the back of the optics box respectively. (C) Sampling mode (two steps). (D) Separation mode (one step).

We chose RB as the analyte, of which the maximum absorption wavelength (545 nm) and the fluorescence wavelength (565 nm) are compatible with that of the light source ($\lambda_{ex} = 532$ nm; $\lambda_{em} = 568.2$ nm) in excitation and emission. Phosphate solution was used as running buffer and sample background electrolyte. In the experiment the adsorption of RB was tried to reduce by adding some organic reagent containing hydrophobic groups into the buffer. We measured 20 μM RB signal when different pH buffers were run that pH range was between 3.5 and 10.5.

Besides, we optimized the separation condition of RB in the phosphate buffer on the hybrid chip. We compared RB signal intensity, migration time and other parameters on different separation conditions. Finally, RB linearity (1–100 μM) and stability ($n = 10$) were studied on the optimized conditions by LIF detection.

2.5. Lysine derivatized with RBITC and detection

In the experiment we chose RBITC as a fluorescent derivatizing reagent and lysine as RBITC-labeled substance.

Lysine solution (100 μM) was prepared individually in 0.1 M sodium tetraborate buffer (pH 9.0). The fluorescent derivatizing reagent, RBITC, was dissolved in DMSO at 5 mM immediately before use. The RBITC solution was then mixed with the lysine solution to give the proper molar ratio of RBITC/lysine (15:2). The reaction proceeded at room temperature overnight in the dark. The resulting RBITC-labeled lysine was diluted with running buffer to a final concentration of about 10 μM prior to injection. Freshly labeled lysine was prepared every 2 weeks and stored at 4 °C when not in use.

At last we tried to separate the labeled mixture solution. The experiment was performed with the optimized buffer.

3. Results and discussion

3.1. RB adsorption on unmodified PDMS channel

In the experiment three-step method was carried to inject and separate the analyte. The results showed the method was effective to achieve good repeatability and reduce peak tailing. At first 10 mM phosphate buffers were used without adding any additives over entire pH range (3.5–10.5). When 20 μM RB injected for 5 s (step 2) to separate and detect, no obvious difference was found between the baseline and the electropherograms with injection under these conditions. When injecting sample for 20 s and after successive runs, the signal had a little increase but no peak observed. Then we replaced the sample with the buffer to clean RB in the separation channel. The result showed that by several cleanings the increased PMT intensity could hardly recover to the blank signal level. These phenomena adequately proved that RB adsorption on the PDMS was very strong. To our knowledge PDMS can adsorb nonpolar hydrophobic analytes, and RB has a hydrocarbon part in its structure, which makes it apt to adsorb on the surface of PDMS in the separation process.

3.2. Solute adsorption of RB to PDMS

It was proposed that when some organic reagent containing hydrophobic groups was added into the buffer, the organic reagent would combine to PDMS. And if most of the hydrophobic sites of PDMS were occupied, the adsorption might be controlled. At first 10 mM phosphate solution of pH 9.5 was as buffer to test. Several organic solvents with low molecular weight, such as CH_3OH, $CH_3CH_2CH_2OH$, $CH_3CH_2OHCH_3$ and CH_3COCH_3, were chosen and added with various concentrations from 5% to 20% (v/v) to the buffer, respectively. However, RB peak was not detected on the hybrid chip, while using glass chip the peak was obvious. This behavior might be due to that these organic reagents are soluble, more hydrophilic than other organic reagents, and not of concentration enough high. Owing to these natural properties and factors, the chosen reagents had less ability to combine with PDMS, and their surface modifications were not stable. Because of these, the combined molecules on PDMS were prone to be replaced by RB and the adsorption is serious yet. So these series of attempts were not successful.

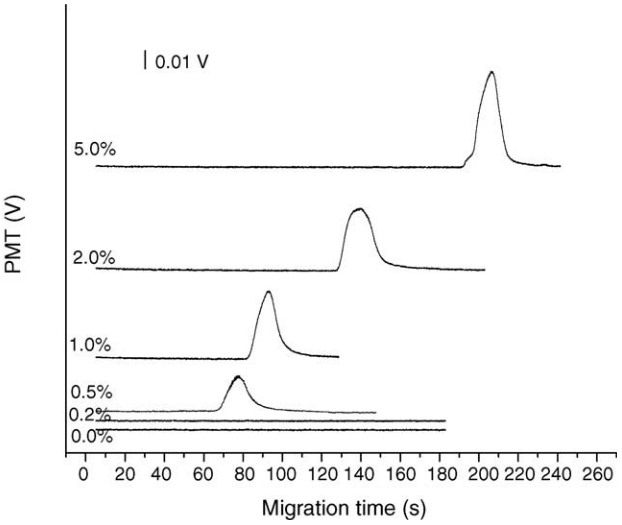

Fig. 2. PMT signal of 20 μM RB in 10 mM phosphate buffer with different concentrations of Triton X-100 (pH 9.5). Sampling voltage mode: step 1 of (pin 3–pin 4) 300 V for 15 s, step 2 of (pin 3–pin 1) 1200 V for 10 s; separating voltage mode: (pin 2–pin 1) 1200 V for 300 s and pin 3/pin 4 1050 V; detection site at about 28 mm from BR. that the first step is applying 300 V to pin 3 and ground to pin 4 (pin 3–pin 4) for 15 s, the second step is applying high voltage to pin 3 and ground to pin 1 (pin 3–pin 1).

By the same idea, we explored the use of surfactant additives instead of those organic solvents to dynamically modify the PDMS microchannels. Triton X-100 of nonionic surfactant adding into the running buffer, we measured the PMT signal of 20 μM RB on the PDMS/glass chip. From Fig. 2, it was observed that When Triton X-100 concentration was added at 0.5% (v/v) or higher, the RB signal was appeared, and the peak shape was satisfactory. Table 1 gave the relationship between RB peak signal and Triton X-100 concentration, such as effective plate number, EOF. EOF was measured by replace each other of sample and run buffer, that sample was prepared in the correspond buffer. The plate number was calculated as $N = 16(t/w)^2$, where w was the peak width at baseline and t was the peak migration time through separation channel. The result showed that the migration time agreed with the EOF. The effective plate numbers were increasing when the concentration of Triton X-100 increased. Generally when the concentration of Triton X-100 was 2.0% or 5.0%, RB signal was better. But taking into consideration peak migration time too long at 5% Triton X-100, 2.0% Triton X-100 was the choice as the optimized concentration.

Besides the PMT signals, the chip-based CE current was also measured when running buffer was added by different concentrations of Triton X-100. And the phenomenon was in agreement with the viscosity of the running buffers that the current was decreasing with the viscosity increasing.

Table 2
Correlative parameters of RB signal at different buffer concentrations[a]

	Buffer concentration (mM)			
	5.0	10.0	15.0	20.0
Peak height (PMT/V)	0.111	0.109	0.093	0.100
Migration time (s)	108.5	153.3	179.7	210.9
Plate number (24 mm)	258	416	379	460

[a] Sampling mode and separation mode as in Fig. 2; detection site at about 28 mm from BR.

3.3. Buffer pH and concentration effects on the RB signal

To our knowledge, buffer pH value is an important factor to CE separation efficiency and peak signal. When the running buffer added with 2% Triton X-100, the result was obtained that at pH 3.50 EOF was minimum, and at pH 9.50 it was at a maximum. This behavior was due to the surface-bound silanol groups of the flat glass. Moreover the investigated result showed that the EOF varied from 1.82 to 3.55 (10^{-5} cm^2/V s) over the pH range from 3.5 to 10.5, and the significant stability between pH 5.5 and 10.5 was good with a 1.84% difference (see Fig. 3[1]). Finally a pH of 9.5 was chosen for the optimized pH of buffer, because at this pH, the peak intensity was the maximum and the sensitivity was high (see Fig. 3[2]).

Furthermore the buffer concentration was also studied that investigated concentration was from 5.0 to 20 mM. When the buffer concentration was less than 10 mM, the current was not over 3 μA. While the concentration was increasing to 15 mM, the current was rapidly rising (>5 μA). And the migration time became longer, that was consistent with Joule heat. Besides this, peak width was broader, and the observation was considered the result of sample zone diffusing. According to the effective plate numbers, 20.0 mM seemed to be a proper concentration (see Table 2). But in the experiment we found that the high current (>5 μA) would affect peak signal stability that the signal intensity (peak height or peak area) decreased or disappeared after a few runs. Furthermore considering the migration time (see Table 2), we finally chose 10.0 mM as the optimized concentration.

Table 1
RB signal parameters at different concentration Triton X-100[a]

	Triton X-100 concentration (%, v/v)					
	0	0.2	0.5	1.0	2.0	5.0
EOF (10^{-5} cm^2/V s)	–	–	3.89	3.74	3.48	2.88
Plate number (24 mm)	–	–	93	209	320	670

[a] Sampling mode and separation mode as in Fig. 2; detection site at about 28 mm from BR.

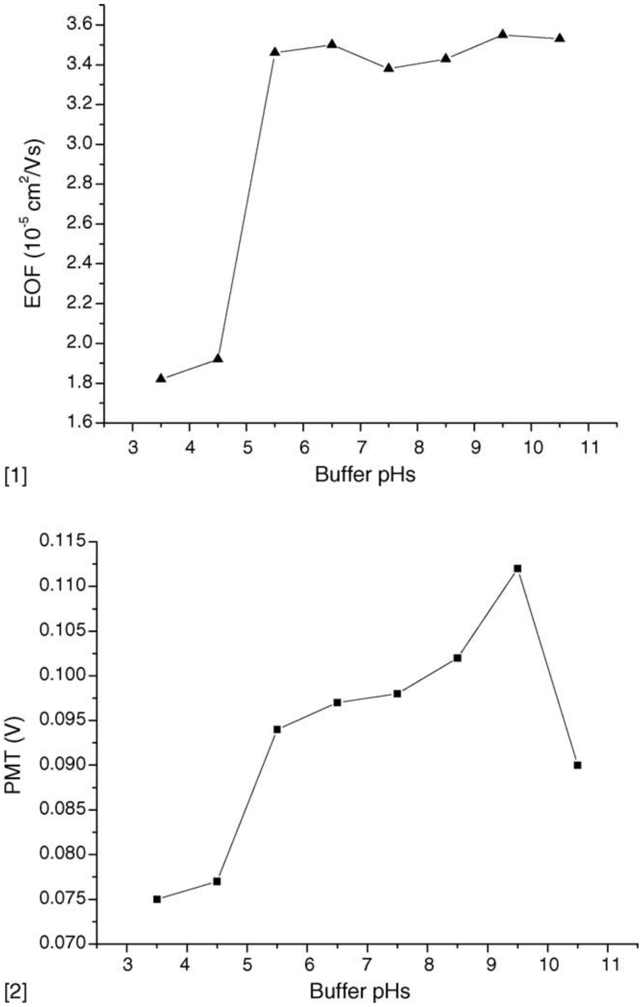

Fig. 3. Optimization of buffer pH. Concentration of buffer 10 mM added 2% Triton X-100. Twenty micromolars of RB prepared in the corresponding buffer as sample. Sampling and separation voltage set as in Fig. 2. [1] EOF value of different pHs (3.5–10.5); [2] RB LIF intensity when different pH buffer running.

3.4. *Influence of separation voltage*

Separation voltage is also important to CE, that will effect on peak width, migration time, etc. In this section, the potentials of pin 3 and pin 4 were changed with the potential of pin 2 in order to ensure the constant current detected at pin 3 and pin 4 ($-0.5 \pm 0.1\,\mu$A). The relationship between current of pin 2/pin 1 and voltage of pin 2 was analyzed. While the potential of pin 2 was increased to 1500 V, the current of pin 2 was nearly up to 6.0 μA in which condition the peak signal stability was poor. When separation voltage was set between 800 and 1200 V, the peak signal was satisfied, and the plate number was calculated (see Table 3).

Table 3
Plate number and plate height of RB peak at different separation voltage[a]

Separation voltage (V)	Plate number (24 mm)
800	823
1000	886
1200	967

[a] Sampling voltage mode: step 1 of (pin 3–pin 4) 200 V for 15 s, step 2 of (pin 3–pin 1) 1200 V for 5 s; detection site at about 28 mm from BR.

Through a serious of optimization, generally 1200 V was chosen as separation voltage, at the same time 1050 V potential was set at pin 3 and pin 4.

3.5. *Relationship of peak signal and detection site on chip*

In order to obtain better separation efficiency and ensure the non-adsorption of RB, the relationship between RB peak

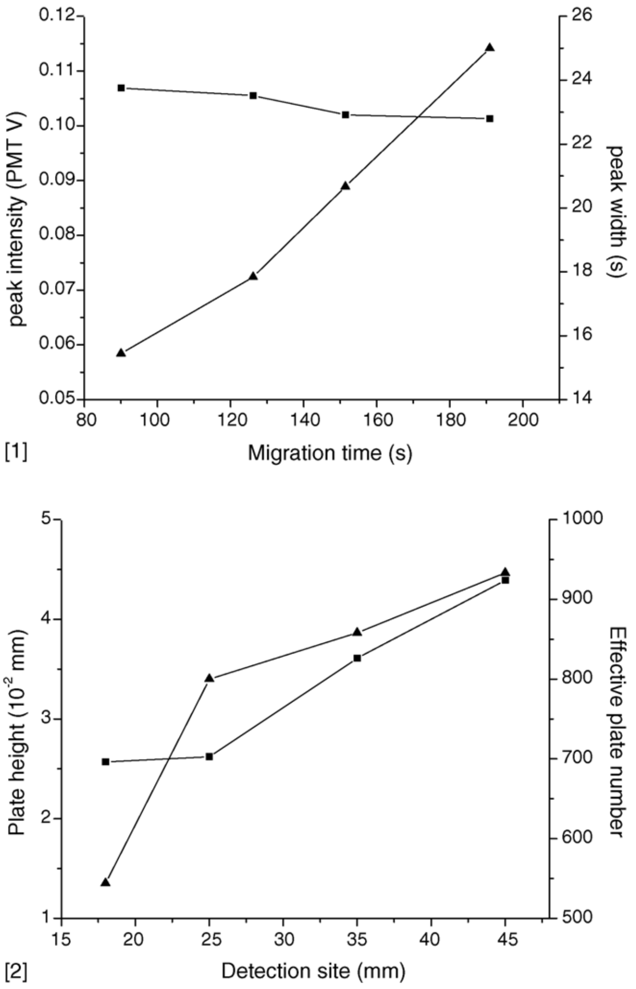

Fig. 4. [1] Twenty micromolars of RB peak intensity and peak width with the corresponding migration time at the different detection site of the chip by LIF (distance from the channel cross: 14, 21, 31, and 41 mm; (■) the relationship between peak intensity and migration time at different site; (▲) the relationship between peak width and migration time). [2] Plate height and effective plate number at different detection distance ((■) the relationship between plate height and detection site; (▲) the relationship between plate number and detection site). Sampling voltage mode: step 1 of (pin 3–pin 4) 200 V for 15 s, step 2 of (pin 3–pin 1) 1200 V for 5 s; separating voltage mode: (pin 2–pin 1) 1200 V for 300 s and pin 3/pin 4 1050 V. Plate height (H) was calculated as $H = L/N$, where L was the distance between the channel cross and the detection point.

signal and detection site was studied. Moreover the plate number and plate height were provided at different detection spot, that the detection spot was at 18, 25, 35 or 45 mm from BR, respectively (see Fig. 4). From the figure we could observe that when increasing the distance of the focused spot the migration time became longer, and the peak width changed broader. The result also showed that the dynamic coating method was satisfied. In order to gain higher effective plate number and make separation resolution better. We chose 45 mm as the detected spot.

3.6. Repeatability and linear relationship

On the optimized conditions, we monitored the reproducibility of 20 μM RB signal ($n = 10$) and measured the relative standard deviation (R.S.D.) of the migration time and the peak height. All measurements were shown in Table 3 and the good reproducibility of migration times and peak intensity was reflected by R.S.D. of 2.62% and 4.78%. The high reproducibility and stability well proved that the optimized condition was beneficial too.

In addition we had achieved linear calibration for RB over a range of 1–100 μM under the optimized conditions. The result showed that the relationship between the intensity based on peak height and the concentration of RB existed in a good linearity. Correlation coefficient achieved at 0.9996, and the limit of detection (LOD, defined as $S/N = 3$) was approximately 0.2 μM.

3.7. Application

In experiments, we performed the labeling reaction at the 15:2 molar ratio of lysine and RBITC, that ratio makes RBITC enough in theory, and then simply separated the mixture of lysine–RBITC and the redundant RBITC. Fig. 5 showed the electropherogram of the 10 μM labeled lysine and the surplus probe. Although the peak signals were weaker, the mixtures were almost separated successfully with 1.2 of resolution. In addition, the base line was always on the blank level during the separation, and this also proved the dynamic coating to be effective.

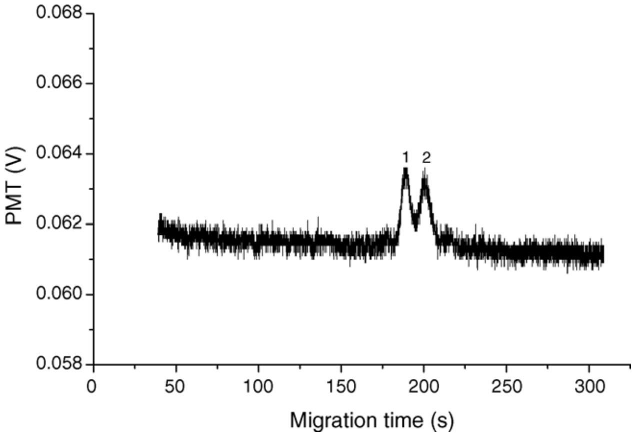

Fig. 5. Electropherogram of 10 μM labeled lysine and surplus RBITC. Detected peaks: RBITC (1), lysine–RBITC (2). Separation buffer 10 mM phosphate with 2.0% Triton X-100 (pH 9.5); sampling voltage mode and separation voltage mode as in Fig. 4; distance of detection spot from BR at about 45 mm.

4. Conclusions

We have demonstrated a simple method to control adsorption of fluorescence dye RB to PDMS with adding 2% Triton X-100 surfactant. According to the experiment results we chose phosphate buffer (pH 9.5) with 2% Triton X-100 as the running buffer. Furthermore, the abundant experiment results about RB have demonstrated that the dynamic coating method was very effective. The electropherogram of derivatized lysine and RBITC probe showed that the present method was applicable for reducing the adsorption of RB-like compounds, and for detection of them on PDMS/glass hybrid chip coupled with LIF.

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