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Simultaneous analysis of seven oligopeptides in microbial fuel cell by micro-fluidic chip with reflux injection mode

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ABSTRACT

In this work, a reflux injection mode for the cross form micro-fluidic chip was studied. This injection mode could flexibly control the length of sample plug from less than one channel width (<83 μm) to tens of channel widths (millimeter-sized) by adjusting the injection time. Namely, the separation resolution or sample detection sensitivity could be selectively improved by changing injection time. Composed of four steps, the reflux injection mode alleviated the electrophoretic sampling bias and prevented sample leakage successfully. On a micro-fluidic chip coupled with laser induced fluorescence (LIF) detector, the injection mode was applied to separate seven oligopeptides, namely GG, GL, RPP, KPV, VKK, WYD and YWS. All analytes were completely separated and detected within 12 min with detection limits of 25–625 nmol/L. At last, the proposed method had been successfully applied to detect oligopeptides consumed by bacillus licheniformis in anode chamber of microbial fuel cell (MFC) to study the effect of oligopeptides on the MFC running.

1. Introduction

The ambitions to transplant a whole laboratory onto a single chip accelerated the advance of technique of micro-fluidic chip (microchip) in recent two decades [1,2]. Integrated with functional units, microchip has the ability to perform functions such as separation and analysis of chemical compounds [3], DNA measurement [4], cell research [5], immunoassay [6], micro-fluidic reactor [7] and so on. Among all of its functions, sample separation with electrophoresis mode is still one of the most important applications for microchip, and has been used widely due to its high efficiency and high speed analysis. The unique injection system for microchip can control the length of sample plug to restrain the peak broadening during separation. Generally, the sample plugs conducted by electrokinetic injection in a cross form microchip are about tens of microns long. So, high separation efficiency can be easily obtained from microchip capillary electrophoresis (MCE) [8]. It is worth mentioning that the high separation efficiency can also be realized in capillary electrophoresis (CE) through sample plug controlling [9]. Although MCE still has some disadvantages such as difficult manipulation with ultra-small sample volumes, higher sensitivity to the sample solvent evaporation and more complicated installment of some detection systems, the application of shorter sample plug injection in microchip is very convenient because the injection components are integrated by nature. Furthermore, the buried channels in microchip have the ability of heat dissipation which can effectively prevent the peak boarding caused by heat.

Electrokinetic injection and pressure injection are the two main injection methods for microchip [10]. In a microchip, sample and buffer solutions are reserved in reservoirs which are connected with each other through micro-channels. So, the pressure pressed on a reservoir can be conducted to the other reservoirs easily during pressure injection, which make solution flow uncontrollable. To perform pressure injection is relative complicated because it usually needs the help of special design of microchip or auxiliary implements such as pump and valve [11–13]. The obvious advantage of electrokinetic injection is its convenience. Solution is driven by electro-osmotic flow which can be controlled just by the setting of high-voltage supply. Proper electric field setting can manage the direction and speed of solution flows without any leakage. So, electrokinetic injection became popular at the start of separation application for microchip [10]. In order to control the shape of sample plug, different geometries of injection channel were studied, such as tee form, twin-tee form, multi-tee form, double cross form and cross form [14–16]. Among these forms, cross form is the most common geometry for electrokinetic injection design. On this simple geometry design, electrophoretic sampling bias for electrokinetic injection can be overcome by prolonging injection time to mobilize even the slowest moving analytes through the intersection.

"Floating injection" is one of the earliest injection modes on cross form microchip. With the floating of buffer and buffer waste reservoirs, the injection voltages are only applied on sample and

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sample waste reservoirs during injection. The defect for "floating injection" is sample diffusion during injection which results in peak broadening [17]. Then the "pinched injection" was proposed to prevent the diffusion of sample plug by adding voltage on buffer and buffer waste reservoirs during "floating injection" [17,18]. Although shorter sample plug can be obtained by "pinched injection" for the purpose of improving the separation efficiency, this injection mode also has its shortcoming that it cannot introduce longer sample plug into separation channel. So, the application of "pinched injection" is limited when a great deal of sample is needed in analysis. "Gate injection" is an injection mode based on continuous sample flow which can control the length of sample plug by changing the dispensing time [19]. But, the "gate injection" suffers a new electrokinetic sampling bias, transradial electrokinetic selection, which occurs as analytes of differing electrophoretic mobilities migrate around the corner of intersection [20]. "Dynamic loading injection" is another injection mode which consists of three steps including "pinched injection". This injection mode can dynamically control the length of sample plug from 2 channel widths (100 μm) to 20 channel widths (millimeter-sized) [21]. Longer length of sample plug adds the amount of injected sample which helps improve detection sensitivity. But, the mode cannot offer shorter sample plug when separation efficiency is emphasized. In this paper, a reflux injection mode was applied on a cross form microchip to separate oligopeptides. This injection mode could flexibly control the length of sample plug from less than one channel width (<83 μm) to tens of channel widths (millimeter-sized) to fit the requirement of separation efficiency or detection sensitivity. Furthermore, the reflux injection was composed with four steps including "pinched injection" which could alleviate the electrophoretic sampling bias by continues sample flow.

Oligopeptides are a series of peptides composed with 2–10 amino acids. Compared with polypeptide and protein, most of oligopeptides are water soluble with smaller molecular mass. Small oligopeptides, similarly as amino acids, can be absorbed by animals directly during intestinal absorption [22]. Oligopeptides also are very important carbon and nitrogen source necessary for the culture of bacteria. Traditionally, HPLC was always employed to separate and determine oligopeptides due to its high selectivity [23,24]. In the past decades, capillary electrophoresis and capillary electrochromatography (CEC) had become more attractive techniques in the oligopeptide analysis [25–32]. In comparison with HPLC, CE and CEC, microchip electrophoresis possesses itself of advantages such as high resolution, less reagent consumption, high speed analysis and so on. So, it is very suitable for the separation and analysis of oligopeptides [33,34]. In this paper, the microchip electrophoresis with reflux injection mode was applied to separate and determine seven oligopeptides in a microbial fuel cell (MFC). A MFC was established and bacillus licheniformis was selected as the microorganisms to generate electrons from its metabolism. Bacillus licheniformis can survive in relatively harsh environment of wide range of temperature and pH value of solution [35]. So it is one of the ideal bacterium for MFC application. Bacteria were placed in anode chamber of MFC and fed with substrate containing oligopeptides. The MFC was run for different time and then the consumption of oligopeptides was analyzed on microchip to study the effect of oligopeptides on the culture of bacillus licheniformis.

2. Experimental section

2.1. Microfabrication

The microchip was fabricated with indium tin oxide (ITO) glass. The surface of ITO glass was coated with a layer of indium tin oxide which acted as sacrificed substrate during wet etching. The photolithographic mask was designed with software (coreldraw 12) and then transferred to a transparent film by laser phototypesetting machine. The ITO glass substrate was cut with dimension 18 mm (length) × 3.5 mm (width) × 1.2 mm (thickness). Prior to fabrication, the surface of ITO glass was wash with liquid detergent and deionized water, respectively. Then the glass was dried in an oven and cool down to the room temperature. The cooled glass substrate was coated with BP218 positive photoresist by a spin coater and then baked at 120 °C for 3 min. After soft baking, the glass substrate was cooled down and covered with the mask to perform UV lithography under the photoetching machine. The development was performed by dipping substrate in 0.5% m/v NaOH for 30 s. After development, the substrate was washed with water to remove NaOH solution and then dipped into 6 M HCl to remove the ITO layer on the pattern. Before wet etching, the back of substrate was pasted with transparent adhesive tape to protect the exposed glass from being etched. Then the wet etching was conducted by dipping the glass substrate into etching solution (5% HF and 20% NH_4F, 30 °C) for etching. After wet etching, the photoresist and ITO layer were removed. Holes with 2 mm diameter were drilled on another cover glass substrate to from sample and buffer inlets. Then the etched substrate and cover substrate were washed and pasted together under water. Lastly, the chip was dehydrated and bond at 560 °C for 20 min.

The injection and separation channels were 83 μm and 116 μm wide, respectively and both of them were 42 μm deep. The distance form sample, buffer, sample waste reservoirs to the intersection all were 1.0 cm and the separation channel was straight form with length 15.3 cm.

2.2. Chemicals and materials

The standards of seven oligopeptides, namely Gly–Gly (GG), Gly-L-Leu (GL), Arg-Pro-Pro (RPP), Lys-Pro-Val (KPV), Val-Lys-Lys (VKK), Trp-Tyr-Asp (WYD), Tyr-Trp-Ser (YWS), were obtained from ChinaPeptides Company (Shanghai, China). Bacillus licheniformis was obtained from Northeast Pharmaceutical Group Shenyang Pharmaceutical Company Limited (Liaoling, China). Photoresist (BP218) was purchased from Kempur Microelectronics (Beijing, China). Analytical grade fluorescein isothiocyanate (FITC) was obtained from Sigma (St. Louis, MO, USA). Boric acid, sodium borate, sodium hydroxide, sodium dodecyl sulfate (SDS) and other reagents were analytical reagent. Bromophenol blue was used to dye the solution for observation. The water used in this experiment was Milli-Q water (18.2 MΩ/cm).

The 1×10^{-2} M stock solutions of each oligopeptides were prepared by dissolving the above oligopeptides in water. FITC was dissolved in the mixture of 99.5% v/v acetone and 0.5% v/v pyridine to obtain 2.6×10^{-2} M stock solution. The derivatization was performed before separation. 10 μL standard solutions of oligopeptides were mixed with 10 μL 2.6×10^{-2} M FITC solution and then adjusted to 100 μL with 10 mM borate buffer (pH 9.18). The mixtures were placed in refrigeratory (10 °C) for 12 h. Before using, proper volume of each derived oligopeptides was mixed and diluted with running buffer to desired concentration. The running buffers ($pH<9.18$) were prepared by mixing the same concentration solutions of sodium borate and boracic acid. The higher pH value ($pH>9.18$) running buffers were prepared from 10 mM sodium borate solution adjusted with 5 M sodium hydroxide solution.

The anode chamber solution for MFC contained phosphate buffer (adjusted with 10 mM NaH_2PO_4 and 10 mM Na_2HPO_4, pH = 7.0), 15 g/L sodium chloride and seven oligopeptides (1×10^{-4} M). The cathode chamber solution was 5 mM potassium ferricyanide.

2.3. Apparatus

The microchip-LIF system had been introduced in previous report [36]. It contained a microchip and a LIF detector (Shandong Normal University, China) having excitation wavelength 473 nm and emission wavelengths 525 nm. The microchip high-voltage supply (Shandong Normal University, China) had an adjustable voltage range between 0 and 5000 V. A chromatography workstation (model HW-2000, Qianpu Software, Shanghai, China) was used to record electropherograms. A photoetching machine (JKG-2A, Shanghai XueZe Optical Machinery Limited Company) was applied to perform UV lithography. A stereoscope was employed to observe the solution flow in micro-channels.

The structure sketch of MFC is shown in Fig. 1. The anode chamber and cathode chamber are columniform with diameter15 mm and height 30 mm. The two chambers were connected with a salt bridge which acted as hydrogen ion channel. Two ITO glasses were used as electrodes at the bottom of the chambers and the bacteria of bacillus licheniformis were cultured on the surface of the ITO glass of anode chamber. The MFC supply power to a resistance (0.5 MΩ) and the applied voltage for the resistance was recorded with a voltmeter.

2.4. Procedure

All solutions were filtered with a 0.22 μm membrane and then ultrasonicated for 3 min before use. After every separation, the micro-channels were washed with 0.1 M NaOH, water and running buffer for 5 min, respectively. The method to separate standard solution mixture of seven oligopeptides was established first.

Two same MFCs (MFC 1 and MFC 2) were established. MFC 1 was added with substrate (containing oligopeptides) and bacillus licheniformis into anode chamber and potassium ferricyanide into cathode chamber. Then the resistance was connected and voltage output was recorded continuously. MFC 2 was prepared like MFC 1. But the substrate in anode chamber of MFC 2 did not contain oligopeptides. The voltage outputs were compared between MFC 1 and MFC 2 to study the effect of oligopeptides on bacillus licheniformis culture. The anode chamber solutions (containing oligopeptides) of MFC 1 were sampled at intervals and analyzed to study the consumption of oligopeptides by bacillus licheniformis. The MFCs were run in room temperature (23 °C).

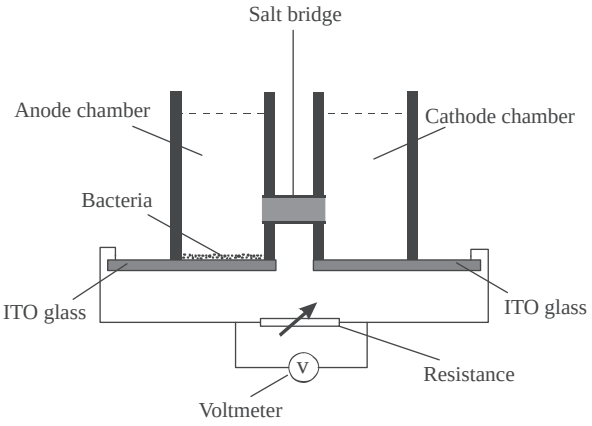

Fig. 1. The structure sketch of MFC.

3. Result and discussion

3.1. Reflux injection mode

The process of reflux injection includes four steps which are illustrated in Fig. 2A with images at left and schematics of the applied voltage and flow direction at right. All of the applied voltages were normalized with respect to the highest voltage 2000 V and represented with the symbol ε marked in the schematics. Buffer reservoir, sample waste reservoir, sample reservoir and buffer waste reservoir were at the direction of 1, 2, 3 and 4, respectively (see step 1). The bromophenol blue solution was used as sample and observed under a stereoscope. A pinched injection was performed first in step 1 to prevent the sample diffusion.

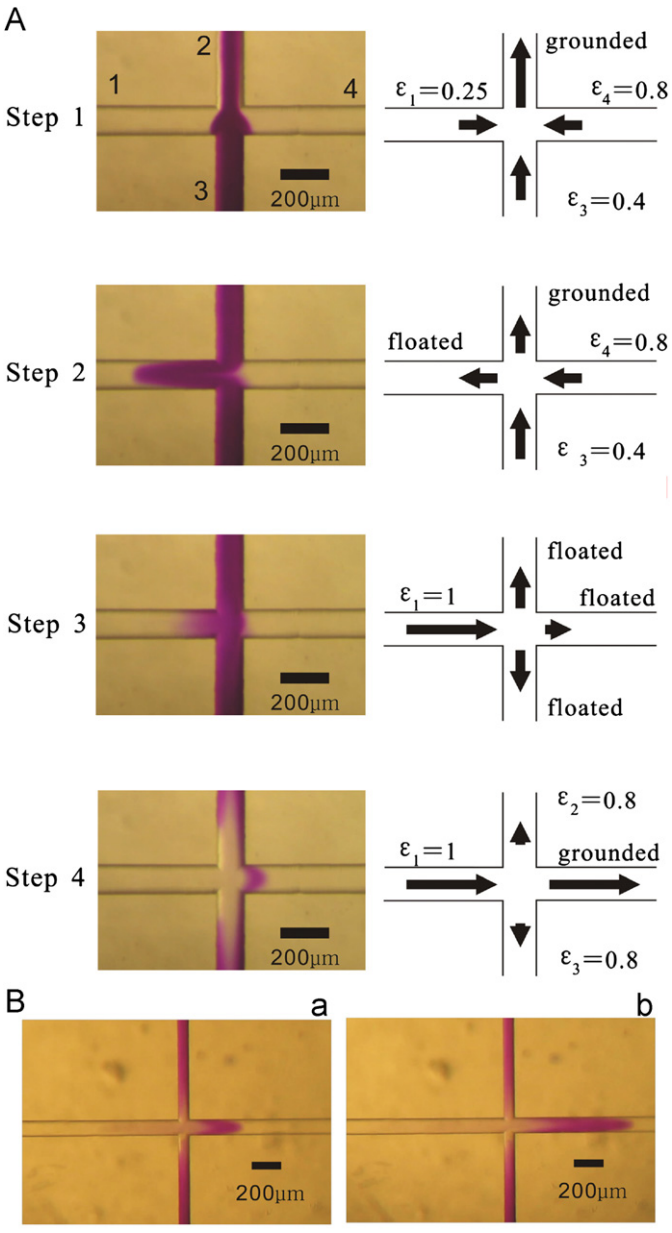

Fig. 2. The four-step process of reflux injection and the images of sample plug. Solution conditions: 10 mM $Na_2B_4O_7$–H_3BO_3 buffer (pH=9.0) including 20 mM SDS; Sample: bromophenol blue; Injection mode: reflux injection mode. A: (T_1=0.2 s and T_2=2 s). B: (a): T_1=0.5 s and T_2=0.5 s, (b): T_1=1 s and T_2=0.5 s.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Electrophoretic sampling bias could be alleviated greatly by prolong the injection time properly. Then the buffer reservoir was floated for a period of time (T_1) in step 2. With the floating of buffer reservoir, a part of sample was driven to the channel of direction 1. The length of this reflux sample plug depended on the span of floating time T_1. For the case of Fig. 2A, the T_1 was 0.2 s. Then in step 3, the buffer reservoir was applied with separation voltage (2000 V, $\varepsilon_1=1$) with the other reservoirs floating for the time T_2. The sample plug was push back to the direction 4 immediately. However, the separation channel (direction 4, 15.3 cm) was much longer than the two channels from intersection to sample (at the direction 3) and sample waste reservoirs (at the direction 2) that sample flow was very difficult to flow into separation channel due to high resistance. So, the sample plug was confined to flow across the intersection to enter the separation channel. For the case of Fig. 2A, the T_2 was 2 s. If the T_2 was too long, most of sample would flow into the channels of direction 2 and 3 that resulted in the shortening of sample plug. In step 4, both the sample reservoir and the sample waste reservoirs were applied with voltage ($\varepsilon_3=\varepsilon_2=0.8$) to resist the separation voltage ($\varepsilon_1=1$) applied at buffer reservoir. Then to enter the channels of direction 2 and 3 became very difficult for sample flow due to electric field resistance. But to enter the channel of direction 4 (namely separation channel) became relatively easy. At last, a part of sample flow was pushed into the separation channel to accomplish sample injection. Except for step 1, the reflux injection mode was quite different from the reported injection method of dynamic loading [21]. The sample was confined to flow across the intersection before step 4, which prevented the diffusion of sample plug. In step 3, the avail of natural fluid resistance of micro-channel distributed the excrescent sample which made it possible to form ultra short sample plug just through adjusting time of T_2. In dynamic loading injection method, the shortest sample plug was about 2 channel widths. But for the case of reflux injection mode in Fig. 2A, the length of sample plug was about 76 μm shorter than the width of channel (83 μm) which made for high separation efficiency for separation. Moreover, the length of sample plug could be adjusted flexibly even to more than ten channel widths by changing two time parameters of T_1 and T_2 (see Fig. 2B). Compared with dynamic loading injection method, the sample shapes of reflux injection were more symmetrical which resulted in symmetrical sample peaks.

FITC was analyzed to study the effect of T_1 and T_2 on the separation and the results are shown in Fig. 3. When $T_1=2$ s and $T_2=0.5$ s, electrophoregram a was obtained with the theoretical plate number 1.10×10^4 for FITC. Then T_1 was decreased to 1 s and $T_2=0.5$ s. Electrophoregram *b* was obtained with the theoretical plate number 1.80×10^4 for FITC. The ratios of peak area and peak width between *a* and *b* were 2.54 and 1.33, respectively. It indicated that the sample plug was shortened and the injection amount of sample was decreased with the decrease of T_1. But the shortened sample plug improved the separation resolution quickly. When $T_1=2$ s and T_2 was increased to 2 s, electrophoregram *c* was obtained with the theoretical plate number 3.12×10^4 for FITC. Compared with electrophoregram *a*, the ratios of peak area and peak width between *a* and *c* were 6.10 and 1.65, respectively. It was obvious that the increase of T_2 shortened the sample plug and decreased the injection amount of sample quickly. Most of sample was distributed into channels of direction 2 and 3 (see step 3). Although the detection sensitivity was decreased, the separation efficiency was increased greatly for electrophoregram *c*. As described previously, longer T_1 and shorter T_2 would prolong the length of injected sample plug and increase the detection sensitivity. Shorter T_1 and longer T_2 would increase the separation resolution greatly at the cost of detection sensitivity.

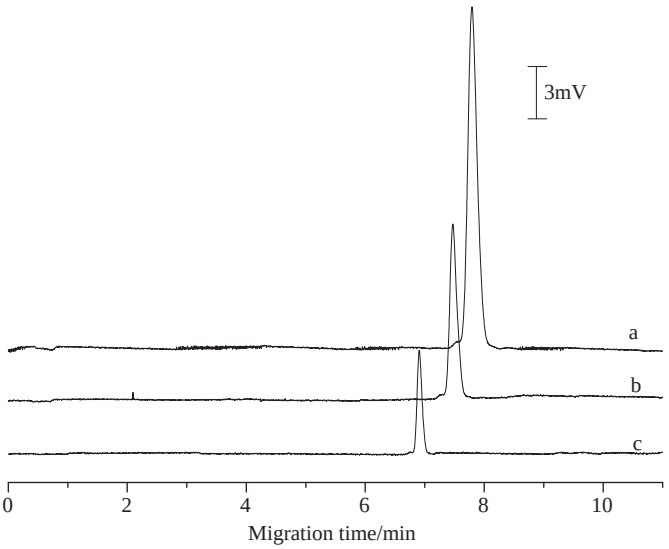

Fig. 3. The effect of T_1 and T_2 on the separation of 5×10^{-6} M FITC. Solution conditions: 10 mM $Na_2B_4O_7$–H_3BO_3 buffer (pH=9.0) including 20 mM SDS; Injection mode: reflux injection mode. a: $T_1=2$ s and $T_2=0.5$ s; b: $T_1=1$ s and $T_2=0.5$ s; c:$T_1=2$ s and $T_2=2$ s.

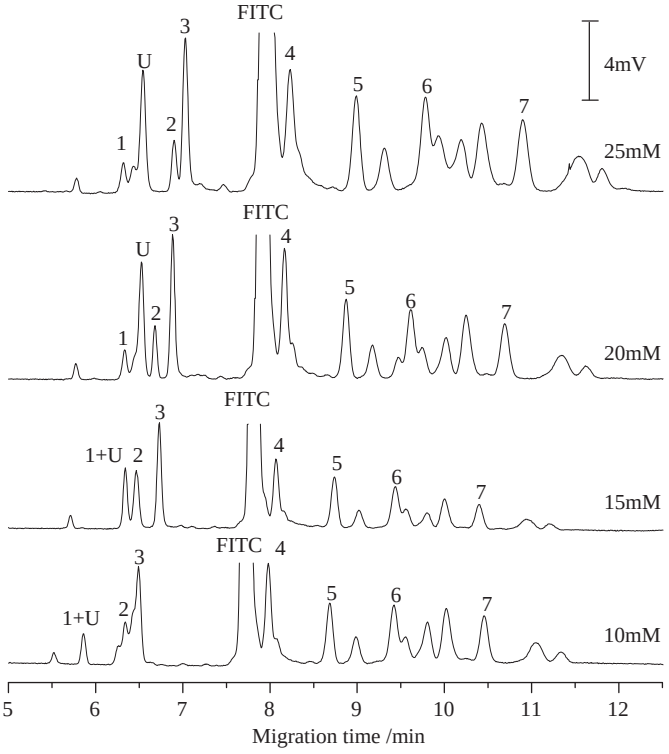

Fig. 4. Influence of the SDS concentration on the electropherogram for oligopeptides. Concentration: all of oligopeptides were 1×10^{-5} M; Solution conditions: 10 mM $Na_2B_4O_7$ buffer (adjusted with 5 M NaOH, pH=9.25); Injection mode: reflux injection mode ($T_1=0.2$ s, $T_2=2$ s), Separation voltage: 2000 V. 1, KPV; 2, VKK; 3, RPP; 4, YWS; 5, GL; 6, GG; 7, WYD; U, unknown peak.

Five consecutive injections of 1.0×10^{-4} M FITC were performed to study the repeatability of reflux injection. The RSD of the peak height and migration time were 4.26% and 0.74%, respectively which indicated good repeatability for the reflux injection mode. Sample leakage was very seldom observed during separation because excrescent sample was distributed into channel 2 and 3 during injection.

3.2. Optimization of separation conditions

3.2.1. Effects of concentration of SDS

First, the target compounds were separated under capillary zone electrophoresis (CZE) mode in phosphate and borate buffer. The result showed that it was impossible for a CZE mode to achieve the complete separation of the seven oligopeptides. In order to achieve better resolution, a micellar electrokinetic chromatography (MEKC) method was developed on microchip and the result is shown in Fig. 4. When SDS concentration was below 20 mM, the sample peaks became lower and three compounds, namely KPV, VKK and RPP, were hard to be separated. Only at the concentration of 20 mM, could the seven oligopeptides be observed with relatively high peaks. With the increase of SDS concentration, the retention time of the analytes is increased and the sample YWS became very close to the peak of FITC. So, 20 mM SDS was selected for the subsequent experiment.

3.2.2. Effects of pH and concentration of buffer solution

Concentration and pH of the running buffer strongly affect the resolution of separation. Therefore, it is vital to select the most suitable concentration and pH value for the running buffer. The dependence of the migration times of the oligopeptides on pH was studied with borate buffer in the pH range 8.75–10.5. The migration times were prolonged with the increase of pH value. At pH 9.0, the migration time of VKK was longer than that of RPP and the better resolution of analytes could be achieved. So, pH 9.0 was found to be the optimum pH value for the running buffer.

The effect of concentration of the running buffer on separation was studied by using 5, 10, 15, 20, 25, 30, and 40 mM borate buffer buffer (pH 9.0). The results showed that oligopeptides could not be separated completely when the buffer concentration was below 10 mM. At the concentration of 10 mM, the seven analytes could be separated completely. When the concentration was increased, the peaks of YWS and FITC became overlapped again. So, 10 mM was chosen as the concentration for running buffer. Under the optimum condition, the electropherogram for the mixture of seven oligopeptides is shown in Fig. 5a.

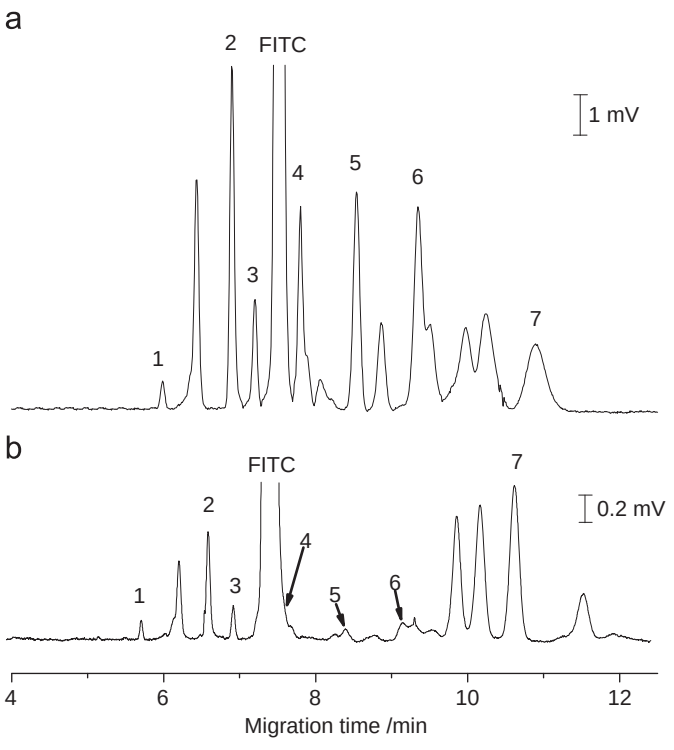

Fig. 5. Electrophoregrams for oligopeptide separation. Solution conditions: 10 mM $Na_2B_4O_7$–H_3BO_3 buffer (pH=9.0) including 20 mM SDS; Injection mode: reflux injection mode (T_1=0.2 s, T_2=2 s), Separation voltage, 2000 V. 1, KPV; 2, RPP; 3 VKK; 4, YWS; 5, GL; 6, GG; 7, WYD. a: 1×10^{-5} M mixed standard solution for oligopeptides. b: anode chamber solution of MFC 1 (6 h).

3.2.3. Linearity and detection limit

In order to determine the linearity of the seven oligopeptides, a series of concentrations of mixed solutions were tested under the optimized conditions. The detection limits were calculated on the basis of an S/N ratio of 3 and the results are listed in Table 1.

3.2.4. Application

In order to compare the effect of oligopeptides on bacillus licheniformis culture, substrate in MFC 1 was added with oligopeptides (1×10^{-4} M) which were not added in the substrate of MFC 2. The two MFC were run to record the change of voltage outputs and the results are shown in Fig. 6. In most time, the voltage output of MFC 1 was higher than that of MFC 2, which indicated higher power output for MFC 1. Moreover, MFC 1 showed better durability than MFC 2 in duration time. It was obvious that bacillus licheniformis fed with oligopeptides had better metabolism. The oligopeptides were consumed by bacillus licheniformis as important carbon and nitrogen source necessary for the culture of bacteria.

Table 1
Regression equations, correlation coefficients, and detection limits.

Compound	Regression equation[a]	Correlation coefficient	Linear range (μmol/L)	Detection limit (nmol/L)
KPV	Y=0.1647X–0.0444	0.9997	1.25–20	625
RPP	Y=1.2476X–0.0902	0.9958	0.31–10	25
VKK	Y=0.2764X–0.0512	0.9985	1.25–20	100
YWS	Y=0.5695X–0.2265	0.9950	0.625–10	50
GL	Y=0.8408X–0.6005	0.9963	0.625–10	50
GG	Y=0.7474X–0.5766	0.9950	0.625–10	50
WYD	Y=0.2530X–0.0544	0.9962	0.625–10	50

[a] Y is the peak height (mV), X is the concentration of analytes (μmol/L).

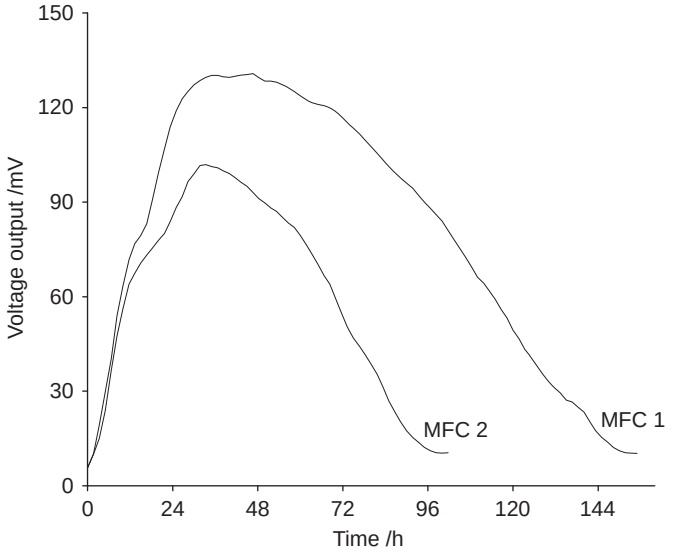

Fig. 6. Voltage outputs of MFC 1 and MFC 2.

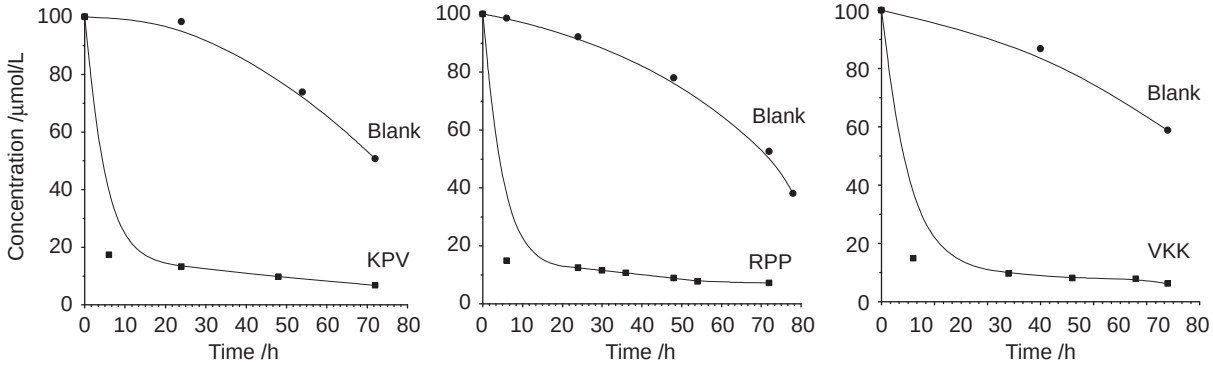

Fig. 7. The trend of concentration change for three oligopeptides in MFC.

The anode chamber solutions (containing oligopeptides) of MFC 1 were sampled at intervals. Then the samples were filtered (to rid solution of bacteria), diluted and derivatized. After derivatization, the sample was analyzed to study the consumption of oligopeptides by bacillus licheniformis. The electropherogram is shown in Fig. 5b. Most of oligopeptides could be observed in the sample of 6 h. But the peak of YWS was overlapped with that of FITC during separation. The GL and GG were consumed quickly and the concentrations became too low to track after 6 h. The peak of WYD would increase slowly after a long time running of MFC, which might be caused by the peak overlapping of metabolites from bacteria. So the changes of concentration for KPV, RPP and VKK were study in the subsequent time. To validate the method, recovery experiments for three oligopeptides were performed by adding 2.00 μmol/L standard solution into MFC sample of 6 h. The recoveries for KPV, RPP and VKK were 110%, 82.0% and 120%, respectively. The trend of concentration change for the three oligopeptides is shown in Fig. 7. The blanks were the concentration of three oligopeptides in reference MFC containing no bacteria. The slight decrease of blank curves might be caused by the slow decomposition of oligopeptides in room temperature (23 °C). Compared with blank, the three oligopeptides were consumed quickly within 6 h and then the concentration decreased slowly after 24 h. In contrast with Fig. 6, the voltage output for MCF 1 was almost increased to the highest value within 24 h and then stayed on a flatform for about 30 h. It was obvious that the consumed oligopeptides maintained the high output of voltage.

4. Conclusion

Using ITO glass, a cross form microchip was fabricated with long straight separation channel. On this microchip, the reflux injection mode was proposed and studied. Compared with reported injection mode, reflux injection was stable, reliable and applicable for separation. It alleviated electrophoretic sampling bias and prevented sample leakage successfully. Moreover, the separation resolution and detection sensitivity could be adjusted by this injection mode. Seven oligopeptides were separated and determined on microchip with reflux injection mode. Then, the method was successfully applied on determination of oligopeptides in anode chamber of MFC to study the consumption of oligopeptides by bacillus licheniformis.

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References

[1] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Science 261 (1993) 895–897.
[2] A. Arun, G. Simone, G.B. Salieb-Beugelaar, J.T. Kim, A. Manz, Anal. Chem. 82 (2010) 4830–4847.
[3] W. Wang, F. -F. Fu, X. -Q. Xu, J. -M. Lin, G. -N. Chen, Electrophoresis 30 (2009) 3923–3938.
[4] H.J. Oh, J.Y. Park, S.E. Park, B.Y. Lee, J.S. Park, S-K Kim, T.J. Yoon, S-H Lee, Anal. Chem. 81 (2009) 2832–2839.
[5] D. Wlodkowic, K. Khoshmanesh, J.C. Sharpe, Z. Darzynkiewicz, J.M. Cooper, Anal. Chem. 83 (2011) 6439–6446.
[6] Y. Liu, H.X. Wang, J.Y. Huang, J. Yang, B.H. Liu, P.Y. Yang., Anal. Chim. Acta 650 (2009) 77–82.
[7] B. O'Sullivan, H. Al-Bahrani, J. Lawrence, M. Campos, A. Cázares, F. Baganz, R. Wohlgemuth, H.C. Hailes, N. Szita, J. Mol. Catal. B: Enzym. 77 (2012) 1–8.
[8] I. Rodriguez, Y. Zhang, H.K. Lee, S.F.Y. Li, J. Chromatogr. A 781 (1997) 287–293.
[9] T. Zhang, Q. Fang, W. -B. Du, J.-L Fu, Anal. Chem. 81 (2009) 3693–3698.
[10] J.M. Karlinsey, Anal. Chim. Acta 725 (2012) 1–13.
[11] Y. Luo, D. Wu, S. Zeng, H. Gai, Z. Long, Z. Shen, Z. Dai, J. Qin, B. Lin, Anal. Chem. 78 (2006) 6074–6080.
[12] H. Hu, X. Yin, L. Qi, J. Liu, Electrophoresis 30 (2009) 4213–4218.
[13] N. Dossi, R. Toniolo, S. Susmel, A. Pizzariello, G. Bontempelli, Electrophoresis 31 (2010) 2541–2547.
[14] C.-H. Tsai, R.-J. Yang, C.-H. Tai, L.-M. Fu, Electrophoresis 26 (2005) 674–686.
[15] L.-M. Fu, R.-J. Yang, G.-B. Lee, H.-H. Liu, Anal. Chem. 74 (2002) 5084–5091.
[16] R.-J. Yang, L.-M. Fu, G.-B. Lee, J. Sep. Sci. 25 (2002) 996–1010.
[17] S.C. Jacobson, R. Hergenroder, L.B. Koutny, R.J. Warmack, J.M. Ramsey, Anal. Chem. 66 (1994) 1107–1113.
[18] S.V. Ermakov, S.C. Jacobson, J.M. Ramsey, Anal. Chem. 72 (2000) 3512–3517.
[19] S.C. Jacobson, R. Hergenroder, A.W.J. Moore, J.M. Ramsey, Anal. Chem. 66 (1994) 4127–4132.
[20] B.E. Slentz, N.A. Penner, F. Regnier, Anal. Chem. 74 (2002) 4835–4840.
[21] D. Sinton, L Ren, D. Li, J. Colloid Interface Sci. 266 (2003) 448–456.
[22] H. Newey, D.H. Smyth, J. Physiol. 152 (1960) 367–380.
[23] M. Shibue, C.T. Mant, R.S. Hodges, J. Chromatogr. A 1080 (2005) 68–75.
[24] J. Lu, M. Ji, R. Ludewig, G.K.E. Scriba, D-Y Chen, J. Pharm. Biomed. Anal. 51 (2010) 764–767.
[25] H. Wan, L.G. Blomberg, J. Chromatogr. A 792 (1997) 393–400.
[26] Y. Yang, R.I. Boysen, J.I-C. Chen, H.H. Keah, M.T.W. Hearn, J. Chromatogr. A 1009 (2003) 3–14.
[27] Y. Yang, R.I. Boysen, M.T.W. Hearn, J. Chromatogr. A 1079 (2005) 328–334.
[28] I. Miksik, P. Sedlakova, J. Sep. Sci. 30 (2007) 1686–1703.
[29] M. Herrero, E. Ibanez, A. Cifuentes, Electrophoresis 29 (2008) 2148–2160.
[30] G.K.E. Scriba, P. Schmitt-Kopplin (Eds.), Capillary Electrophoresis, Humana Press, Totowa, NJ, 2008.
[31] V. Kasicka, Electrophoresis 31 (2010) 122–146.
[32] V. Kasicka, Electrophoresis 33 (2012) 48–73.
[33] S. Zhao, Y. Huang, M. Shi, J. Huang, Y.-M. Liu, Anal. Biochem. 393 (2009) 105–110.
[34] N.A. Lacher, N.F. Rooij, E. Verpoorte, S.M Lunte, J. Chromatogr. A 1004 (2003) 225–235.
[35] A. Palop, J. Raso, R. Pagan, S. Condon, F.J. Sala, Int. J. Food Microbiol. 29 (1996) 1–10.
[36] Z. -W. Wang, W. -J. Wang, W. Wang, L. -J. Xu, G. -N. Chen, F. -F. Fu, J. Sep. Sci. 34 (2011) 196–201.