Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 36 (2004) 1–8
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

# A novel double coating for microemulsion electrokinetic chromatography with laser-induced fluorescence detection: as tested with amino acid derivatives

Jianping Xie [a,b], Xiaofeng Chen [a], Jiyou Zhang [a], Jiaqin Liu [a,c], Jianniao Tian [a], Xingguo Chen [a], Zhide Hu [a,*]

[a] Department of Chemistry, Lanzhou University, Lanzhou 730000, PR China
[b] Department of Chemistry, Zhoukou Teachers College, Henan 466000, PR China
[c] Department of Chemistry, Mianyang Teachers College, Sichuan 621000, PR China

Received 8 March 2004; received in revised form 12 May 2004; accepted 17 May 2004

Available online 27 July 2004

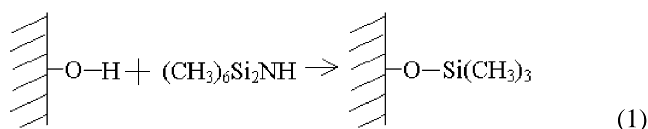
**Abstract**

A novel double coating (DC) was developed for fast and reproducible microemulsion electrokinetic chromatography (MEEKC), as tested with separation and determination of amino acids using laser-induced fluorescence (LIF) detection after derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol. The simple coating is a combination of a removable covalent layer and a dynamic SDS coating. Hexamethyldisilazane was utilized for the covalent layer that can be regenerated on-line. Compared with previous no-coating method, the analysis time was shortened; and the reproducibility of migration times was improved.
© 2004 Elsevier B.V. All rights reserved.

*Keywords:* Dynamic coating; Double coating; Hexamethyldisilazane; Microemulsion electrokinetic chromatography; Laser-induced fluorescence; Amino acids

## 1. Introduction

The most ordinarily used wall coatings for modifying the charge on the capillary surface are the following: (nondynamically) covalently bonded/cross-linked polymer [1–3], (noncovalently) adsorbed cationic polymers [4–5], (dynamically and noncovalently) adsorbed surfactants [6–8], and dynamic double coating (DC) [9–12]. Nondynamic coating often needs very long coating time. Common dynamic coating often uses polymers or surfactants with complicated molecular structure and then may be troublesome for mass spectrometric detection due to high background signals. Hexamethydisilazane (HMDS) with simple molecular structure is a commonly used protective reagent for hydroxyl groups in organic synthesis [13–14]. Under a mild condition, HMDS reacts with hydroxyl to form trimethylsilyl protective group. The trimethysilyl group can be removed under another mild condition as necessary. It was just based on this principle that we tried a trimethylsilyl coating. The reaction equation can be expressed as following:




(1)

Microemulsion electrokinetic chromatography (MEEKC) has been shown to be useful for the analysis of very lipophilic substances and very hydrophilic substances, as well as pharmaceuticals and natural products [15–18]. In addition, capillary electrophoresis (CE) with laser-induced fluorescence (LIF) detection has received many attentions since high detection sensitivity can be achieved [19–22]. In this paper, we firstly tried a novel double coating, which included the trimethylsilyl coating, as tested with MEEKC–LIF of 10 amino acids after derivatization with 4-chloro-7-nitrobenzo-2-oxa-1, 3-diazol. To our

* Corresponding author. Tel.: +86 931 8912540; fax: +86 931 8912582.
*E-mail addresses:* huzd@lzu.edu.cn, enjoyskies@163.com (Z. Hu).

0731-7085/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2004.05.004

knowledge, the migration time R.S.D. values in this work are the smallest for CE determination of amino acids [23–25].

## 2. Experimental

### 2.1. Apparatus

All separations were carried out on a P/ACE5510 system (Beckman Coulter Instrument, Fullerton, CA, USA) equipped with a LIF detector. The excitation light from an argon ion laser (3 mW) was focused on the capillary window by means of a fiber-optic connection. The excitation was performed at 488 nm and a 520 nm band-pass filter was used for emission. The system was controlled by P/ACETM station software. The separation was carried out on a 47 cm (40 cm to the detector) × 75 μm i.d. fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China). In MEEKC (no DC) separation, the capillary was treated prior to its first use by flushing with 1.0 M HCl for 20 min, 0.5 M NaOH for 20 min and distilled water for 15 min. Between MEEKC (no DC) runs, a rinse-cycle, 1 M HCl for 1 min, 0.5 M NaOH for 1 min, distilled water for 2 min and run buffer for 2 min was used. In MEEKC–DC separation, a coating procedure was described subsequently. The capillary was maintained at 25 °C. Sample was injected by applying a pressure of 0.5 psi (1 psi = 6894.76 Pa) for 5 s.

### 2.2. Materials

Standard amino acids were obtained from Gansu Institute for Control of Pharmaceuticals, China. NBD-Cl was the product of Tokyo Kasei Kogyo CO, LTD (Japan). HMDS (99%, w/w) was provided by National Laboratory of Applied Organic Chemistry (Lanzhou, China) and was diluted directly 45 times with acetonitrile to form stock HMDS solution. Compound Amino Acid Injection (a pharmaceutical preparation) was obtained from Kang'erjia Pharmaceutical Store (Lanzhou, China). All other reagents were of analytical reagent grade. The 4 mM stock solutions of amino acids were prepared in distilled water, respectively and stored at room temperature. A 34.3 mM stock solution of NBD-Cl was prepared in acetonitrile and stored at 4 °C.

### 2.3. Preparation of the microemulsion and electrolyte

The stock microemulsion was prepared by mixing heptane (3.24%, w/w), SDS (13.24%, w/w), butanol (26.44%, w/w) and distilled water (57.08%, w/w). The run buffers were prepared by mixing the stock microemulsion and 100 mM $Na_2B_4O_7$ solution. And the 40 mM stock derivatization $Na_2B_4O_7$ buffer was prepared from 100 mM $Na_2B_4O_7$ and its pH was adjusted to 8.4 with 1.0 M HCl.

### 2.4. Sample preparation

An artificial amino acid mixture sample was prepared by mixing these amino acids. The concentration of each one is 0.19 mM, respectively.

After 100-fold-diluted with distilled water, Compound Amino Acid Injection (a pharmaceutical preparation) was directly derivatizated for analysis.

### 2.5. Derivatization procedure [22]

In 1.5 ml plastic tubes, 40 μl standards or samples with 360 μl NBD-Cl solutions were added to 600 μl derivatization $Na_2B_4O_7$ buffer. The blank solutions were prepared by mixing 600 μl derivatization buffer, 40 μl distilled water and 360 μl NBD-Cl solution. Then, the mixtures were kept in a hot-water-bath to react 60 min at 60 °C. Prior to the analysis, the derivatization solutions were diluted with 40 mM derivatization buffer (pH 8.4) to the desired concentrations.

### 2.6. Coating procedure

New capillaries were treated prior to its first coating by flushing with 1.0 M HCl for 20 min, 0.5 M NaOH for 20 min, distilled water for 15 min, acetonitrile for 5 min, HMDS solution for 10 min, respectively. At the beginning of each day, the capillary was rinsed with 0.5 M NaOH for 2 min, distilled water for 2 min, acetonitrle for 3 min, HMDS solution for 3 min, respectively. At the end of each day, the capillary was rinsed with 0.5 M NaOH for 1 min, distilled water for 2 min, acetonitrile for 2 min, respectively. Capillaries were stored dry with the ends kept in acetonitrile. Between two runs, a rinse-coating-cycle, distilled water for 1 min, acetonitrile for 1 min, HMDS solution for 2 min and run buffer for 2 min was used after optimized.

### 2.7. Calculations

In this method, there is no neutral marker for electroosmotic flow, so the electroosmotic flow mobility is calculated by abrupt change of current proposed by Li et al. [26].

$$\mu_{\mathrm{eof}} = \frac{L^2}{V((t_1 + t_2)/2)} \quad (2)$$

where $L$ is total length of the capillary; $V$ is the separation voltage; $t_1$ and $t_2$ is the start and end time of the abrupt change of current.

## 3. Results and discussion

### 3.1. MEEKC (no DC) of amino acid derivatives

We have described the details of derivatization and MEEKC separation conditions of nine basic or neutral amino acids in another paper [22]. In present experiments, the

conditions were used directly for obtaining the data of the three late eluting amino acids (Tyr, Glu and Asp). Under the conditions the three amino acid standards were derivatizated and separated. It was observed that the separation of the three amino acids resulted in very long times with poor reproducibility. Further investigation also indicated that no linear relationships could be obtained between the concentrations of the three amino acids and the corresponding peak heights.

### 3.2. Optimization and evaluation of HMDS DC procedure

Before MEEKC–DC separation, new capillaries were treated prior to its first use by flushing with 1.0 M HCl, 0.5 M NaOH, distilled water, acetonitrile, and HMDS solution, respectively. Between MEEKC–DC runs, a rinse-coating-cycle was introduced including a distilled water rinse, an acetonitrile rinse, an HMDS coating and a run buffer rinse. To obtain reproducible separation, the rinse-coating-cycle was investigated.

Firstly, the rinse solvent was selected among methanol, ethanol and acetonitrile. It was observed that acetonitrile produced more reproducible separation than the others. Secondly, rinse time for every rinse liquid was studied. It was found that different rinse time of distilled water or acetonitrile or run buffer hardly affected the migration time of the analytes. Thirdly, the effect of the coating time of HDMS solution was investigated in the range 0.2–5.0 min. It was found that the coating time of HDMS solution hardly effected the migration time of the analytes, either. But the coating time of HMDS solution also must be controlled accurately, because the numbers of reproducible runs varied with the change of the HDMS coating time. It was observed that 2 min provided the most runs (at least 21 runs here; the EOF of the consecutive 21 runs is shown subsequently) although every coating time provided at least five runs with similar reproducibility (R.S.D. ($n = 5$) of migration time $< 0.2\%$). So 2 min was selected as the optimum coating time. Fourthly, The EOF was also studied when no coating was performed. It was observed that the EOF decreased when the coating did not perform. Table 1 shows the EOF and separation time data of fifteen consecutive runs. From the table, it can be found that the EOF can maintain its value with different coating times (except zero). The EOF decreases when the coating time is zero. The EOF value decreases 7.85% after only three runs. The increased EOF can be regenerated when the normal rinse-coating-cycle is performed again. This also indicates that the covalent layer in DC can be removed, and DC including the covalent layer and the SDS layer can be reproduced on-line. Finally, between two runs, a rinse-coating-cycle, distilled water for 1 min, acetonitrile for 1 min, HMDS solution for 2 min and run buffer for 2 min was used.

Table 1
EOF data vs. different coating time of the consecutive 15 runs[a]

| Run | Coating time (min) | $\mu_{eof}$ ([a]$10^{-4}$ cm$^2$ V$^{-1}$ s$^{-1}$) | Separation time (min) |
|---|---|---|---|
| 1 | 2 | 7.01 | 4.35 |
| 2 | 0 | 6.57 | 4.57 |
| 3 | 0 | 6.52 | 4.69 |
| 4 | 0 | 6.46 | 4.77 |
| 5 | 2 | 6.97 | 4.37 |
| 6 | 2 | 7.00 | 4.35 |
| 7 | 2 | 7.02 | 4.36 |
| 8 | 1 | 7.01 | 4.35 |
| 9 | 1 | 7.02 | 4.34 |
| 10 | 4 | 7.01 | 4.35 |
| 11 | 4 | 7.01 | 4.35 |
| 12 | 0 | 6.56 | 4.57 |
| 13 | 0 | 6.48 | 4.73 |
| 14 | 2 | 7.00 | 4.36 |
| 15 | 2 | 7.01 | 4.35 |

[a] The separation time is equal to the migration time of Asp derivative.

Fig. 1 displays the EOF (anode to cathode) with consecutive 21 runs. Electrophoretic buffers consisted of 40 mM

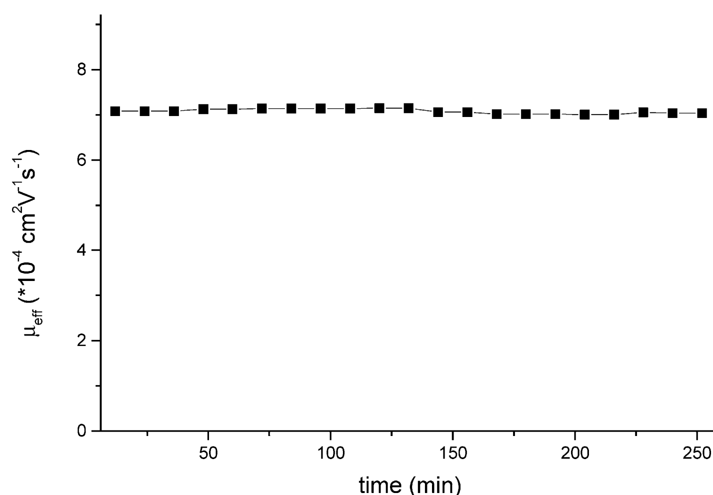



Fig. 1. Coating reproducibility as reflected by the EOF with consecutive 21 injections. *Separation conditions*: 40 mM $Na_2B_4O_7$, 16% microemulsion (v/v), pH 9.6, applied voltage 20 kV, the length of the capillary is 47 cm (40 cm to the detector), and sample injection time 5 s with a 0.5 psi pressure. The optimized rinse-coating-cycle was performed between runs.

Table 2
Regression data, R.S.D. and detect limits of the amino acids ($n = 5$)

| Amino acids | Regression equations[a] | Ranges (μM) | Correlation coefficients | R.S.D. (%) | | LOD (nM) |
|---|---|---|---|---|---|---|
| | | | | Time | Height | |
| Arg | $Y = 0.1817x - 0.02389$ | 0.06–8.0 | 0.9977 | 0.14 | 5.50 | 0.33 |
| Pro | $Y = 0.1708x - 0.00948$ | 0.06–8.0 | 0.9995 | 0.15 | 4.79 | 0.31 |
| Val | $Y = 0.02428x - 0.00123$ | 0.06–8.0 | 0.9996 | 0.13 | 4.54 | 0.25 |
| Leu | $Y = 0.02366x - 0.00083$ | 0.06–8.0 | 0.9999 | 0.15 | 4.14 | 0.26 |
| Ala | $Y = 0.0848x - 0.01039$ | 0.06–8.0 | 0.9980 | 0.14 | 3.96 | 0.55 |
| Phe | $Y = 0.2063x - 0.01756$ | 0.06–8.0 | 0.9990 | 0.15 | 4.47 | 0.25 |
| Gly | $Y = 0.1788x - 0.01872$ | 0.06–8.0 | 0.9986 | 0.15 | 4.40 | 0.29 |
| Tyr | $Y = 0.00611x + 0.00043$ | 0.06–8.0 | 0.9996 | 0.15 | 4.02 | 0.63 |
| Glu | $Y = 0.1628x - 0.01492$ | 0.06–8.0 | 0.9986 | 0.15 | 4.46 | 0.33 |
| Asp | $Y = 0.1175x - 0.01674$ | 0.06–8.0 | 0.9977 | 0.18 | 5.73 | 0.48 |

[a] $Y$ is the peak height (RFU) while $x$ is the concentration of corresponding amino acid (μM).

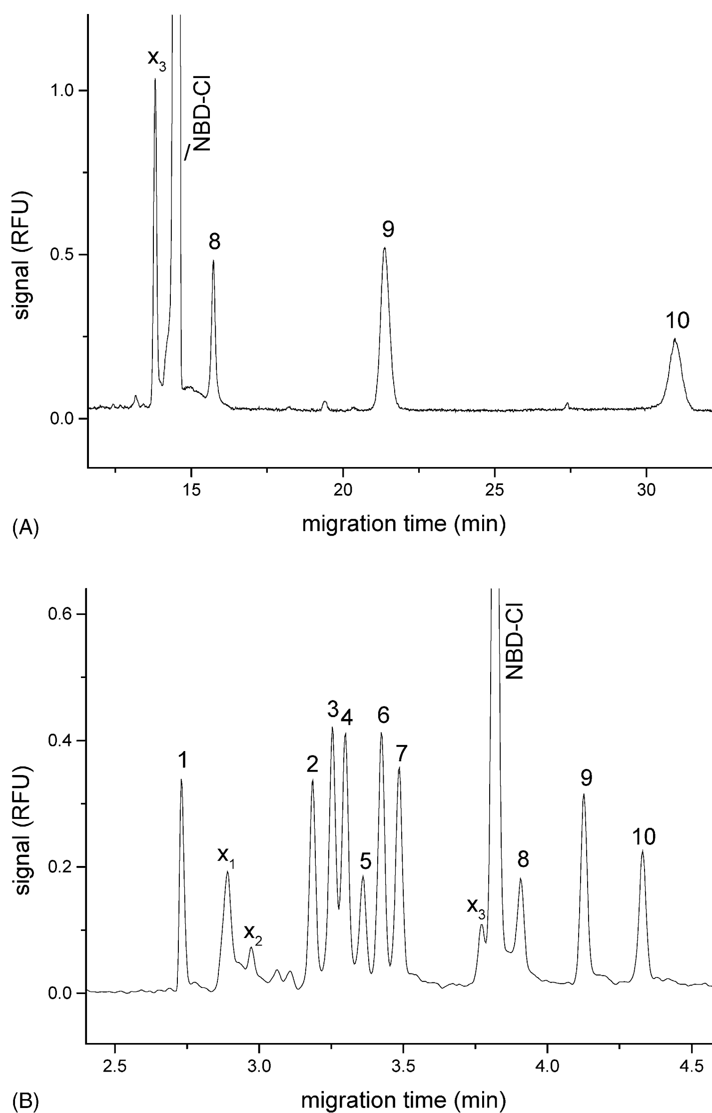



Fig. 2. Electophoregrams for the standards and samples: (A) for the standards using no DC; (B) for the standards using DC; (C) for the artificial mixture sample using DC; (D) for Compound amino acid injection using DC. Peak identification: (1) Arg; (2) Pro; (3) Val; (4) Leu; (5) Ala; (6) Phe; (7) Gly; (8) Tyr; (9) Glu; (10) Asp; $x_1 - x_3$ are unidentified peaks. *Separation conditions*: 40 mM $Na_2B_4O_7$, 16% microemulsion (v/v), pH 9.6, applied voltage 20 kV, the length of the capillary is 47 cm (40 cm to the detector), and sample injection time 5 s with a 0.5 psi pressure.

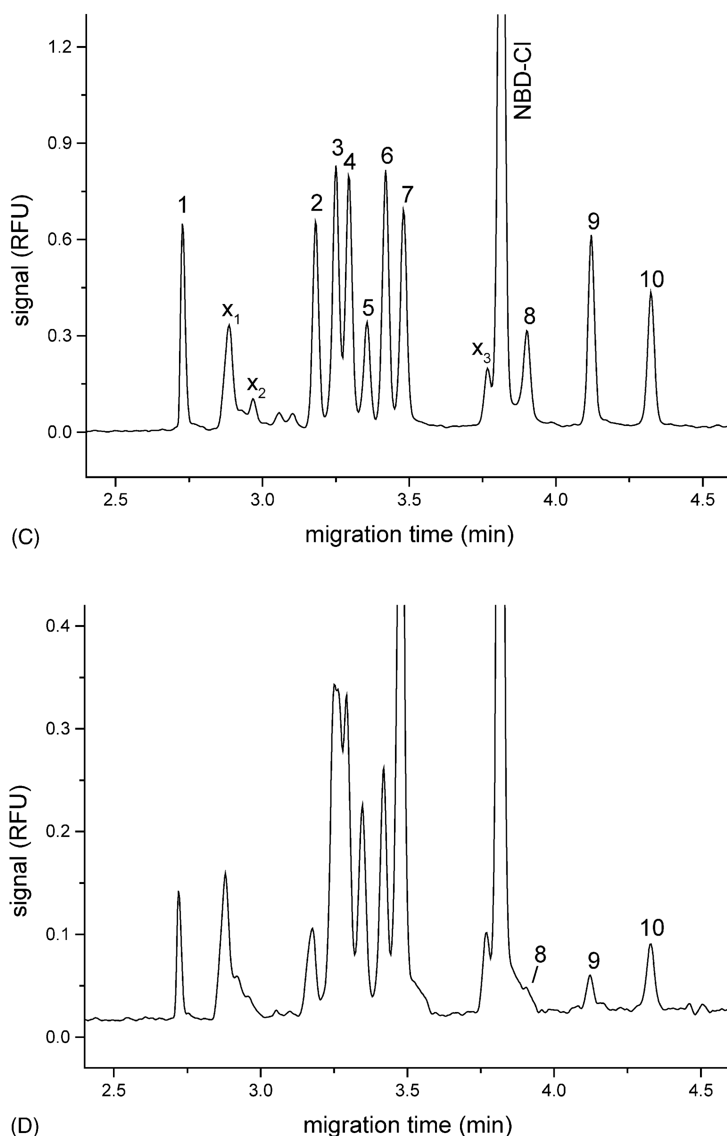



Fig. 2. (*Continued*).

$Na_2B_4O_7$ and 16% microemulsion at pH 9.6. Excellent reproducibility was observed in 21 runs (12 min for each run). Comparing the maximum and the minimum, the EOF value varied only 2% over 252 min of this test. This indicates that the EOF is increased compared to a bare fused-silica capillary and that the EOF is reproducible for at least 21 repeated injections, and also indicates that the maximum of EOF with respect to coating time of 2 min is about 7.1 $cm^2 V^{-1} s^{-1}$ here.

### 3.3. MEEKC–DC of amino acid derivatives

The separation conditions were 40 mM $Na_2B_4O_7$, 16% microemulsion (v/v), applied voltage 20 kV, just as used above. The buffer pH under the conditions was also determined to be 9.6. In ordinary MEEKC separation (Fig. 2A, $\mu_{eof} = 3.67 \times 10^{-4}\ cm^2 V^{-1} s^{-1}$), better separation resulted in longer analysis time as achieved above. When the coating procedure was added, the analysis time was shortened (Fig. 2B) due to the increasing of electroosmotic flow ($\mu_{eof} = 7.11 \times 10^{-4} cm^2 V^{-1} s^{-1}$). Furthermore, because the trimethylsilyl group is more hydrophobic than a silanol group the adsorption of SDS to the capillary surface is increased resulting in an increasing zeta potential. Under the conditions all of the amino acid standards were separated although Val, Leu and Tyr were not baseline separated.

The effect of microemulsion concentration (expressed as volume percentage of the stock microemulsion added to the run buffer) on EOF in this method was investigated in the range of 0–20%. The results are shown in Fig. 3. The figure indicates that microemulsion of low

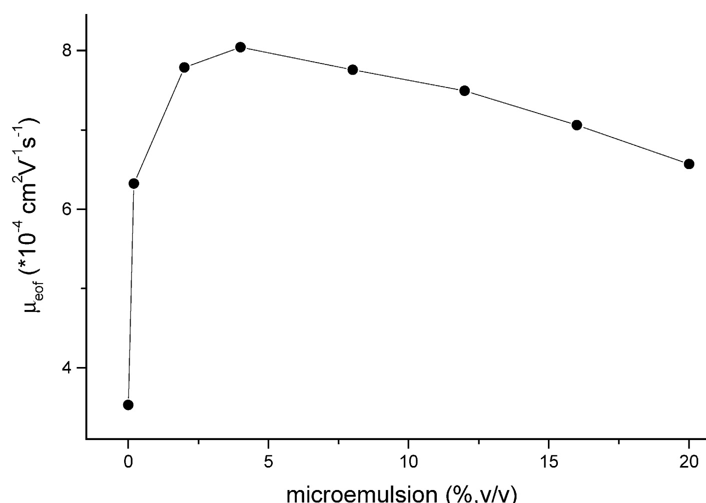



Fig. 3. Effect of microemulsion concentration on EOF. Other conditions, see Fig. 2.

concentration can increase EOF greatly due to the formation of SDS layer. The 4% microemulsion produces the maximum EOF and obtains the shortest separation time. Then EOF decreases slowly with the increasing of microemulsion maybe due to the increasing of ionic strength of the run buffer.

### 3.4. Linearity and reproducibility

Under the selected conditions, the linear relationships between the concentration of the ten amino acids and the corresponding peak heights were investigated. Because Val, Leu, and Tyr derivatives were not baseline separated, the second derivative electrophoregrams were applied. Although no linearity for Tyr, Glu and Asp can be obtained in MEEKC–LIF, good linearity for all the amino acids can be obtained in MEEKC–DC–LIF. The linear relationships for seven amino acids of their concentrations and the corresponding peak heights of the normal electrophoregram (see Fig. 2B) and for three amino acids in the second derivative electrophoregram (see Fig. 4A) are shown in Table 2. The method was validated for the reproducibility of the migration times and the peak heights. The relative standard deviations ($n = 5$) of the migration time and peak height of the three analytes were 0.13–0.18 and 3.96–5.73%, respectively. The detection limits (LOD, based on signal-to-noise, S/N = 3) of the components were also given.

Compared with MEEKC–LIF (no DC), MEEKC–DC–LIF shows many advantages including the improvements of reproducibility, speed, and sensitivity. The comparison results are illustrated in Table 3. From the table it is found that the reproducibility in MEEKC–DC–LIF was much improved over that in MEEKC–LIF; high sensitivity can be obtained within shorter separation time; the sensitivity of three tail analytes in MEEKC–DC–LIF were several times improved over that of MEEKC–LIF.

### 3.5. Applications

The samples were injected. All the ten amino acid derivatives in the artificial mixture sample and the three late eluting amino acids in Compound Amino Acid Injection were separated under the selected conditions described above. The electrophoregrams of the real samples were shown in Fig. 2C and D. From the figures it was observed that all the analytes were capable to be determined combined with the second derivative electrophoregrams because they were separated and had their own peaks although Val, Leu, Tyr were not baseline separated. Therefore, Arg, Pro, Ala, Phe, Gly, Glu and Asp in both of the samples were determined by the normal electrophoregrams, while Val, Leu and Tyr in the artificial mixture sample were determined by the second derivative electrophoregram (Fig. 4B). Tyr in Compound Amino acid Injection was not determined just because its peak was too low. The analytical results were summarized in Table 4. The recovery of the method was determined with the addition of the standard substances in the real samples. Table 4 also shows that the result values were satisfactory.

Table 3
Comparison data between using no DC and using DC[a]

| Amino acids | Time (min) | | R.S.D. of time (%) | | LOD (nM) | |
|---|---|---|---|---|---|---|
| | no-DC | DC | no-DC | DC | no-DC | DC |
| Arg | 5.72 | 2.73 | 0.56 | 0.14 | 0.60 | 0.33 |
| Pro | 8.21 | 3.19 | 0.76 | 0.15 | 0.42 | 0.31 |
| Val | 8.63 | 3.26 | 0.72 | 0.13 | 0.32 | 0.25 |
| Leu | 8.92 | 3.31 | 0.63 | 0.15 | 0.47 | 0.26 |
| Ala | 9.39 | 3.36 | 0.71 | 0.14 | 0.87 | 0.55 |
| Phe | 9.92 | 3.44 | 0.75 | 0.15 | 0.56 | 0.25 |
| Gly | 10.6 | 3.49 | 0.69 | 0.15 | 0.54 | 0.29 |
| Tyr | 15.7 | 3.94 | 5.18 | 0.15 | 1.99 | 0.63 |
| Glu | 21.4 | 4.15 | 2.67 | 0.15 | 1.76 | 0.33 |
| Asp | 30.9 | 4.35 | 3.55 | 0.18 | 3.98 | 0.48 |

[a] The no-DC data except Tyr, Glu and Asp comes from our previous work [22].

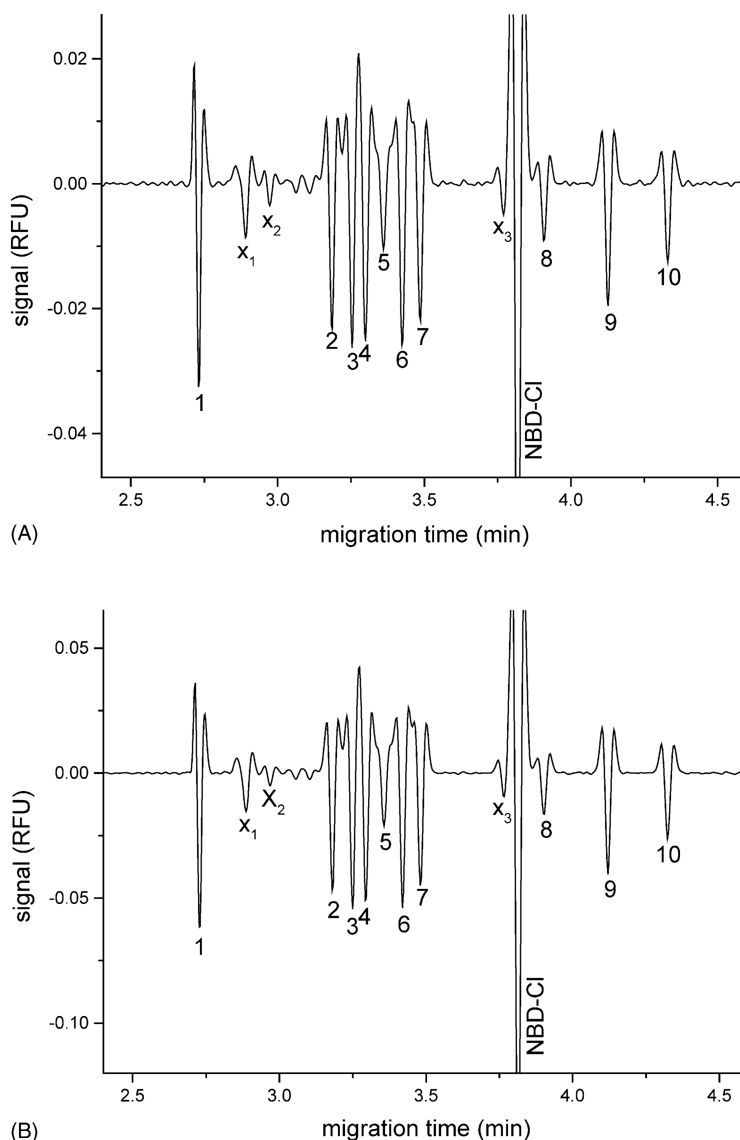



Fig. 4. Second derivative electrophoregrams for the standards and sample: (A) for the standards using DC; (B) for the artificial mixture sample using DC. Peak identification, see Fig. 2.

Table 4
Determination of the amino acids in the samples ($n = 3$)[a]

| Amino acids | The artificial mixture | | Compound Amino Acid Injection | |
|---|---|---|---|---|
| | Content (mM) | Recoveries (%) | Content (μg/ml) | Recoveries (%) |
| Arg | 0.180 | 94.7 | | |
| Pro | 0.191 | 100.5 | | |
| Val | 0.202 | 106.3 | | |
| Leu | 0.199 | 104.7 | | |
| Ala | 0.184 | 96.8 | | |
| Phe | 0.193 | 101.6 | | |
| Gly | 0.187 | 98.4 | | |
| Tyr | 0.193 | 101.6 | | |
| Glu | 0.186 | 97.9 | 1.07 | 95.0 |
| Asp | 0.183 | 96.3 | 2.33 | 96.1 |

[a] The determination of Val, Leu and Tyr is combined with second derivative electrophoregram.

## 4. Conclusions

MEEKC separation often results in long analysis time with poor reproducibility. The new DC can provide a stable and increased EOF, and then lead to short separation time, excellent reproducibility and high sensitivity. With the use of the new DC, MEEKC–DC–LIF was developed successfully to separate and determine the tested amino acids after derivatization with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazol.


## Acknowledgements

The financial support of National Nature Science Foundation of Chinese (No. 20275014) is gratefully acknowledged.

## References

[1] J.K. Towns, F.E. Regier, J. Chromatogr. 516 (1990) 69–78.
[2] G. Kleindienst, C.G. Huber, D.T. Gjerde, L. Yengoyan, G.K. Bonn, Electrophoresis 19 (1998) 262–269.
[3] H. Wan, M. Ohman, L.G. Blomberg, J. Chromatogr. A 924 (2001) 59–70.
[4] Y.J. Yao, K.S. Khoo, M.C.M. Chung, S.F.Y. Li, J. Chromatogr. A 680 (1994) 431–435.
[5] E. Cordova, J. Gao, G.M. Whitesides, Anal. Chem. 69 (1997) 1370–1379.
[6] N.E. Baryla, C.A. Lucy, Anal. Chem. 72 (2000) 2280–2284.
[7] J.E. Melanson, N.E. Baryla, C.A. Lucy, Anal. Chem. 72 (2000) 4110–4114.
[8] J.E. Melanson, N.E. Baryla, C.A. Lucy, Trends Anal. Chem. 20 (2001) 365–374.
[9] K.D. Altria, J. Pharm. Biomed. Anal. 31 (2003) 447–453.
[10] C. Lanz, U. Marti, W. Thormann, J. Chromatogr. A 1013 (2003) 131–147.
[11] G. Vanhoenacker, F. de l'Escaille, D. De Keukeleire, P. Sandra, J. Pharm. Biomed. Anal. 34 (2004) 595–606.
[12] C. Lanz, W. Thormann, Electrophoresis 24 (2003) 4272–4281.
[13] S.M. Xing, Y.L. Wang, Silicone Synthetic Technology and Product Application, Chemical Industry Press, Beijing, 2000.
[14] T.W. Greene, P.G.M. Wuts, Protective Groups in Organic Synthesis, third ed., John Wiley & Sons, Inc., New York, 1999.
[15] H. Watarai, Chem. Lett. 231 (1991) 391–394.
[16] K.D. Altria, J. Chromatogr. A 892 (2000) 171–186.
[17] H. Wan, L.G. Blomberg, J. Chromatogr. A 875 (2000) 43–88.
[18] G.B. Li, X.G. Chen, M.C. Liu, Z.D. Hu, Analyst 123 (1998) 1501–1505.
[19] M. Molina, M. Silva, Electrophoresis 23 (2002) 3907–3921.
[20] J.Y. Zhang, J.P. Xie, X.G. Chen, Z.D. Hu, Analyst 128 (2003) 369–372.
[21] J.Y. Zhang, J.P. Xie, J.Q. Liu, J.N. Tian, X.G. Chen, Z.D. Hu, Electrophoresis 25 (2004) 74–79.
[22] J.P. Xie, J.Y. Zhang, H.X. Liu, J.Q. Liu, J.N. Tian, X.G. Chen, Z.D. Hu, Biomed. Chromatogr., in press.
[23] S. Hu, P.C.H. Li, J. Chromatogr. A 876 (2000) 183–191.
[24] J. Zhao, D. Chen, N.J. Dovichi, J. Chromatogr. 608 (1992) 117–120.
[25] J.Y. Zhang, J.N. Tian, J.Q. Liu, H. Gao, X.G. Chen, Z.D. Hu, Mikrochim. Acta 143 (2003) 241–244.
[26] Q.F. Li, X.Y. Zhang, H.Y. Zhang, X.G. Chen, M.C. Liu, Z.D. Hu, Chin. J. Chem. 19 (2001) 581–587.