Journal of Chromatography A, 1217 (2010) 2182–2186

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Controlling of band width, resolution and sample loading by injection system in a simple preparative free-flow electrophoresis with gratis gravity

Jing Shao[a], Si Li[a], Wei Zhang[a], Liu-Yin Fan[a,*], Cheng-Xi Cao[a,**], Ren Sun[b], Yu-Chao Dong[a]

[a] Laboratory of Analytical Biochemistry & Bio-separation, Key Laboratory of Microbiology of Educational Ministry, School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China
[b] School of Naval Architecture, Ocean and Civil Engineering, Shanghai Jiao Tong University, Shanghai 200240, China

ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form 26 January 2010
Accepted 29 January 2010
Available online 10 February 2010

Keywords:
Free-flow electrophoresis
Gravity
Band width
Resolution
Injection
Sample loading

ABSTRACT

In this paper, the controllable band width, resolution and sample loading were investigated by the injection system of free-flow electrophoresis (FFE) with gratis gravity. Two general injection methods were described herein. The first method was the one in which sample injection fluxes were variable with constant background flux, while the second was the one in which the background fluxes were flexible with stable sample flux. With methyl green and crystal violet as two viewable model compounds, a series of experiments were performed, and the experimental results revealed that (1) the sample band width could be under desiring control through the regulation of ratios between sample and background fluxes, (2) the separative resolution could be also adjusted elaborately via the regulation of flux ratios during the separation of methyl green and crystal violet with only one charge disparity, and (3) the sample loading could be conveniently controlled via the flux ratios and an approximate maximum sample loading could be selected under the condition of just completed separation of two adjoining solutes. In addition, it was observed that the flux ratio had soft influence on the separative resolution of two solutes. These results were of significance to the designs on band width, resolution and sample loading in the newly developed FFE device with gratis gravity.

1. Introduction

Free-flow electrophoresis (FFE) has become one of the useful techniques for preparative separation of numerous chemical and biological samples, such as small organic and inorganic ions, amino acids, peptides, proteins, DNA fragments, membrane vesicles, organelles and cells [1–3].

Numerous achievements have been obtained in FFE. In 1937, Tiselius [4] performed moving boundary electrophoresis in free solution. After that, Philpot [5] and Barrolier et al. [6] realized the merits of continuous electrophoresis and designed a FFE device without any support carriers. Hannig [7] in 1961 developed the antetype of current FFE with multi-channel pumps. Kolin and Cox [8] designed a nice continuous-flow electrophoresis in serpentine liquid columns. To well design FFE, Clifton [9], Grateful and Lightfoot [10], Ivory [11,12], Ostrach [13], Rhodes et al. [14] and Strikler and Sacks [15] developed a series of mathematical models and performed relevant simulations on separation in FFE. Kasicka et al. [3,16,17] advantageously revealed the relationship between capillary electrophoresis (CE) and FFE. Thormann et al. [18,19] and Weber and co-workers [20,21] have made a series of investigations on the design of FFE apparatus and techniques, which resulted in the birth of Octopus FFE, a commercial one. Roman and Brown [22] in 1994 and Kriváková et al. [2] gave comprehensive reviews on the preparative FFE.

In 1975–1977, Hannig et al. [23,24] developed a FFE technique for analytical purpose, and the development of numerous FFE-on-chips should be attributed to the great works by Raymond et al. [25], Kohlheyer et al. [26], Kobaysshi et al. [27] and Zhang and Manz [28], Fonslow et al. [29,30], and so on. Recently, Kohlheyer et al. [31] gave a comprehensive review on analytical FFE devices, their applications in biology and biomedicine.

However, the application of FFE was hampered by sample distortion and broadening mainly influenced by five factors, viz., diffusion, hydrodynamic broadening, electromigration dispersion, electrohydrodynamic distortion and Joule heating [25]. To improve the resolution of FFE, Hannig et al. [23], Huebner and Lawson [32], Strickler [33] commenced to study the theoretical relationship between the band width and the ratios of sample to background flux under the condition of $B_0 \ll 2d_z$ (where B_0 was the band width and $2d_z$ was the chamber thickness). Recently, Bauer [34], Glukhovskij

* Corresponding author.
** Corresponding author at: School of Life Science and Biotechnology, Shanghai Jiao Tong University, 800 Dongchuan Rd, Shanghai 200240, China. Tel.: +86 21 34205820; fax: +86 21 34205820.
E-mail addresses: lyfan@sjtu.edu.cn (L.-Y. Fan), cxcao@sjtu.edu.cn (C.-X. Cao).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.01.085

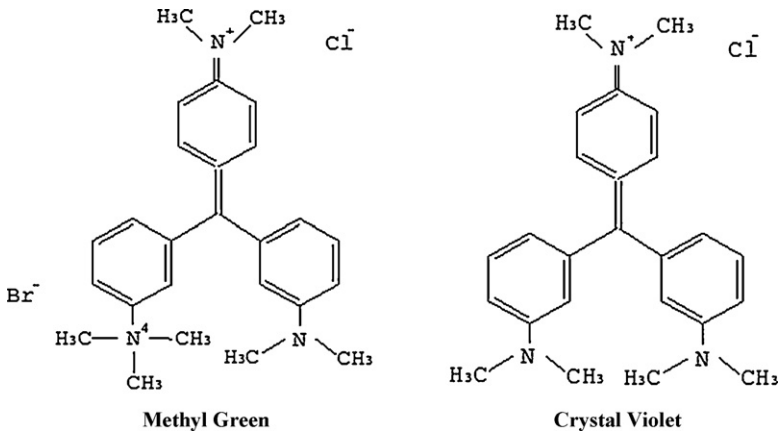

Fig. 1. Chemical structures of the two compounds of methyl green and crystal violet.

[35] and Spanik [36] performed excellent works on enantiomers separation under the similar condition of $B_0 \ll 2d_z$.

In previous work, we [37] developed a novel FFE device with gratis gravity to carry out the separation of three amino acids and sample staking by moving reaction boundary (MRB) [38–40]. The advantages of our FFE apparatus included (i) smooth flow in FFE chamber with the help of gravity and gas cushion injector (GCI), (ii) flexible injection mode from any one of 48 inlets, (iii) simple maintenance and assembly, and (iv) low cost. However, it was difficult to apply the previous injection mode for the study on sample injection in the novel FFE device, as the sample width was equal to or greater than the thickness of FFE chamber, namely there was the condition of $B_0 \geq 2d_z$ rather than the previous condition of $B_0 \ll 2d_z$ in our experiments [23,32–36].

Herein, we focused on the flexible injection mode in the novel FFE, and investigated the effect of ratios between sample and background fluxes on the band width, maximum sample loading and separative resolution.

2. Materials and methods

2.1. Chemical reagents

Methyl green (85% in purity, with 12% methyl violet, Fig. 1) were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). Formic acid and ammonium persulfate (analytical reagent grade, AR) were purchased from Shanghai Chemical reagent Co. (Shanghai, China). Acrylamide and bis-acrylamide were bought from Fluka (Switzerland). TEMED was from Sigma (USA). All solutions were dissolved with ultrapure water (0.055 μS/cm) unless otherwise noted.

2.2. Instrument

The FFE device with gratis gravity has been described in detail in Ref. [37]. Fig. 2 was the actual integration of the device with some modifications. The main modifications were that the glass bottles of self-balance collector (SBC) were replaced by the plastic ones, the lengths of electrode cavities were shortened a little, and two glass baffles were set at the ends of up plate with three holes in each baffle as a newly cooling system. The size of whole device (70 cm × 85 cm × 45 cm) was greatly reduced in contrast to the previous version (80 cm × 100 cm × 160 cm) [37].

A CE system (ACS 2000, Beijing Cailu Instrumental Co., Beijing, China) was used to analyze the samples collected from the separation via novel FFE device (Fig. S1). The CE system was equipped with a power supply which could provide constant voltage up to 30 kV, a HW-2000 chromatography workstation, and a UV-visible detector (190–740 nm, set at 575 nm) as well. A fused-silica capillary (the Factory of Yongnian Optical Fiber, Hebei, China) with 75 μm ID, 54 cm total length, 45 cm effective length was used for dye separation. The capillary was conditioned by rinsing with 1.0 M NaOH for 10 min, ultrapure water for 10 min, 1.0 M HCl for 10 min, ultrapure water for 10 min and running buffer for 30 min in order before analysis of the first sample. Later, the capillary was rinsed with running buffer for 10 min between two different runs.

2.3. Preparation of buffer and samples

There were three kinds of buffers used for FFE and capillary zone electrophoresis (CZE). Taken account of the relative lower Joule heating induced by lower concentration of background electrolyte, the 10 mM pH 3.0 formic buffer was used as the background buffer for the separation of two dyes in FFE and CZE. It was observed that the pH differences of 10 mM pH 3.0 formic buffer between inlets and outlets was about pH 0.1–0.3/tube indicating fair buffer ability under the given conditions. The stocking solution of 100 mM pH 3.0 formic buffer was prepared at first. Before use, the stocking buffer was diluted 10-fold as the background buffer. The electrode buffer used in FFE electrode vials was 30 mM pH 3.0 formic buffer, which was also prepared by the dilution of 100 mM pH 3.0 formic buffer. The sample solution with 425 μg/ml methyl green and 60 μg/ml crystal violet was prepared with the background buffer.

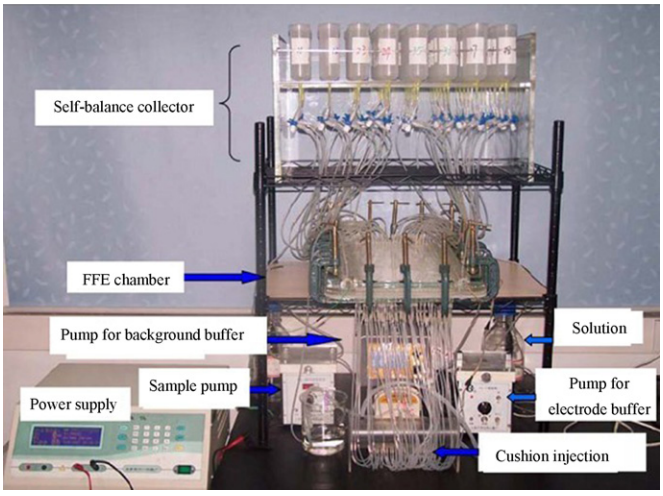

Fig. 2. The integration system of the improved FFE device with gratis gravity [37].

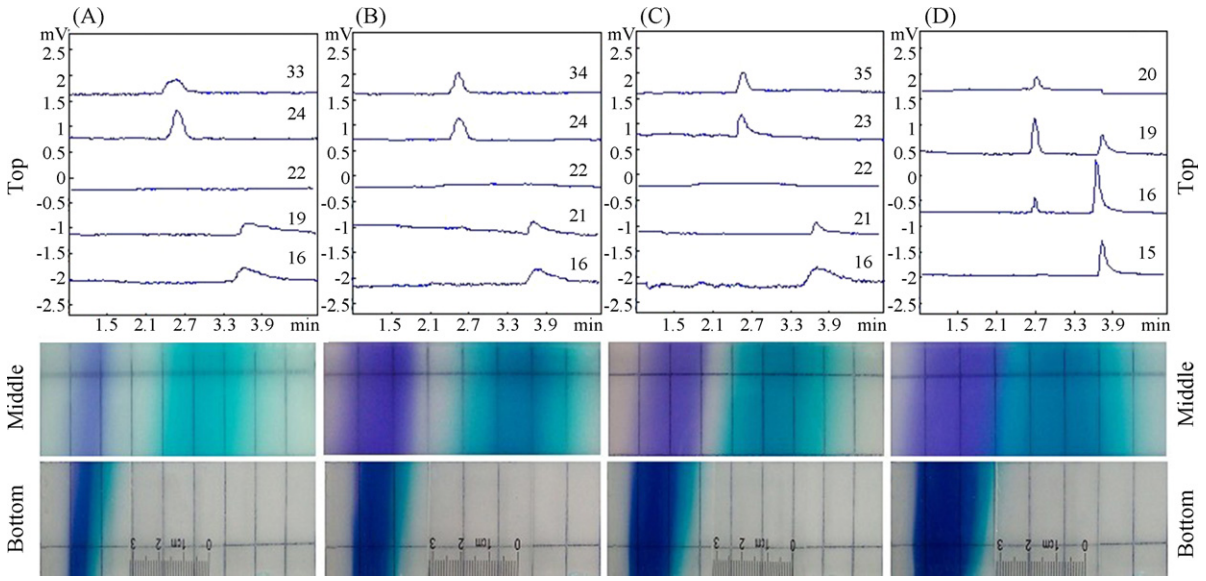

Fig. 3. Effects of ratios between sample and background fluxes on the separation of two dyes under different sample fluxes of A 4× 23.1, B 8× 23.1, C 12× 23.1 and D 16× 23.1 μl/min per inlet. Conditions: background flux = 2× 23.1 μl/min per inlet, sample injection via the 4th inlet, 400 V. The conditions of CZE were given in Section 2.5. Bottom: sample bands just after entering the chamber; middle: sample bands just out off the chamber; top: CZE electrophoregrams of separated samples.

2.4. Procedure of sample loading and resolution control by injection

A high-voltage power supply (DYY-12C, Beijing, China) was used to yield 400 V across the FFE separation chamber. By setting different fluxes of sample and background solutions, various separation efficiencies and maximum sample loading could be achieved under the condition of just complete separation of two solutes. In experiment, the sample solution was injected into the separation chamber via the 4th inlet, while the rest of forty-seven inlets were used for background solution.

2.5. Procedure for CZE of two solutes

A CE was applied to determine the sample solutions collected by self-balance collector (SBC) after the finish of FFE separation (Section 3). The running buffer for the determination of two dyes was 10 mM pH 3.0 formic buffer. The collected sample solutions were directly injected into the capillary without any pretreatment. The injection conditions were 13 mbar pressure and 10 s injection time. The other conditions were: 15 kV, 575 nm wavelength, 75 μm ID × 375 μm OD (54 cm total length and 45 cm effective length), and 25 °C air cooling.

3. Results and discussions

3.1. Resolution control by injection

It was convenient to choose dyes (Fig. 1) to investigate the influence of ratios between sample and background fluxes on the separative resolution of FFE [41]. The band resolution (R_s) was defined as the ratio of the central distance of two adjoining samples

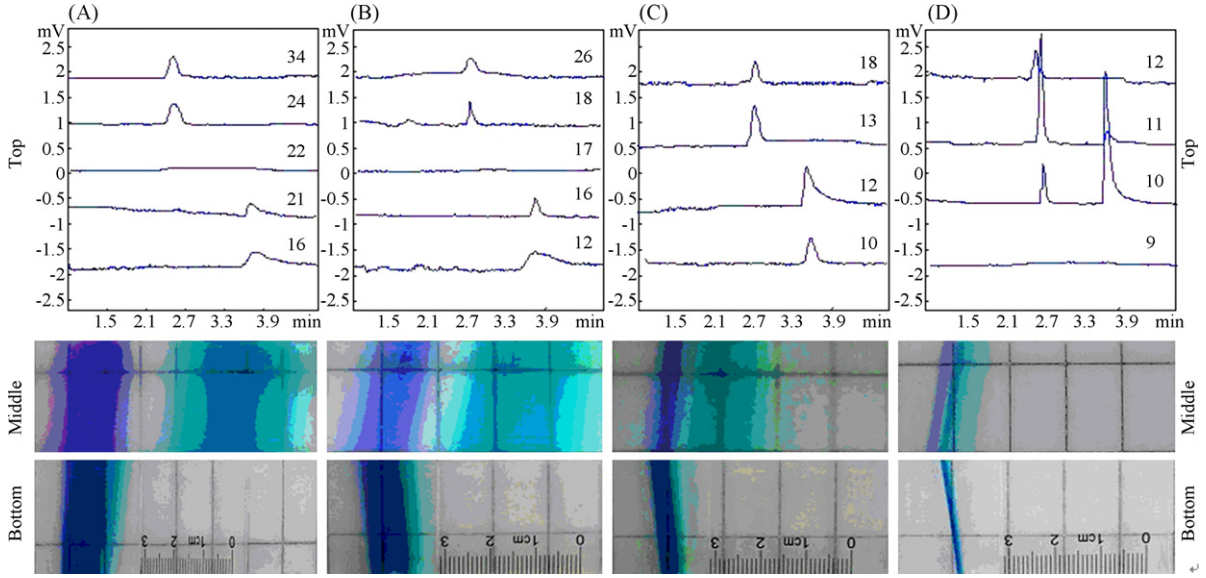

Fig. 4. Effects of ratios between sample and background fluxes on the separation of two dyes under different background fluxes of A 2× 23.1, B 4× 23.1, C 8× 23.1 and D 40× 23.1 μl/min per inlet. Conditions: sample flux = 8× 23.1 μl/min per inlet via the 4th inlet, 400 V. Bottom: sample bands just after entering the chamber; middle: sample bands just out off the chamber; top: CZE electrophoregrams of separated sample.

Table 1
Sample loading and separation resolution R_s of two bands of dyes under different flux ratios of sample to background solutions in Figs. 3 and 4.

Panel	V_s[a] (μl/min/tube)	V_b[b] (ml/min)	Ratio[c] ($\times 10^3$)	R_s	T[d] (min)	Loading (ml/24 h)
3A	92.4	2.2	42.0	1.8	25.2	133.1
3B	184.8	2.2	84.0	1.4	25.2	266.1
3C	277.2	2.2	126.0	1.2	25.2	399.2
3D	369.6	2.2	168.0	0.7	25.2	532.2
4A	184.8	2.2	84.0	1.4	25.2	266.1
4B	184.8	4.3	43.0	1.3	12.7	266.1
4C	184.8	8.7	21.2	1.2	6.3	266.1
4D	184.8	43.4	4.3	0.9	1.3	266.1

[a] V_s was the sample flux via one inlet (from the 4th inlet).
[b] V_b was the background flux via 47 inlets.
[c] The ratio was defined as V_s/V_b viz., the flux ratio between sample and background solution.
[d] T was the residence time of the injected sample and background buffer.

to the half of the sum of the two sample band widths.

$$R_s = \frac{2 \times (d_1 - d_2)}{W_1 + W_2} \quad (1)$$

where, d_1 and d_2 were the central positions of sample 1 and 2, respectively, W_1 and W_2 were the band widths of sample 1 and 2, respectively.

Fig. 3 showed the results on the separation of methyl green and crystal violet by the FFE device under the conditions of constant background flux (=2× 23.1 μl/min per inlet) and variable sample flux (4–16× 23.1 μl/min per tube). In Fig. 3, the bottom exhibited the initial sample bands, the middle displayed the separation of samples just out off the chamber and the top revealed the CZE electrophoregrams of collected samples in that the sequence numbers were the same as that of outlets.

Fig. 3 demonstrated the well control of separative resolution between methyl green and crystal violet via different sample fluxes under constant background flux. As shown in Panel A, the methyl green (No. 24-33) was separated from the crystal violet (No. 16-19) thoroughly if sample flux is 4× 23.1 μl/min. Panel B revealed that the methyl green (No. 24-34) was also separated from the crystal violent (No. 16-21) fully if 8× 23.1 μl/min. Panel C proved that there was only one vacuous tube (viz., No. 22) segregating the methyl green (No. 23-35) and crystal violet (No. 16-21) if 12× 23.1 μl/min. Panel D manifested a poor separation of the two dyes under the sample flux of 16× 23.1 μl/min. The experiments in Fig. 4 verified that the separative resolution of the two dyes could be regulated properly via the adjustment of background fluxes under constant sample flux.

The resolution (R_s) of the two dyes in Figs. 3 and 4 was calculated with Eq. (1) (see Table 1). As shown in Table 1, when the sample loading was doubled (133–266.1 ml/24 h), the resolution was only decreased from 1.8 to 1.4, and the resolution was slightly reduced from 1.4 to 1.2 when the loading was enhanced to 399.1 ml/24 h. Table 1 further demonstrated that the resolution was softly lowered from 1.4 to 0.9, as the background flux increased from 2.2 to 43.4 ml/min, which implied the gentle influence of background flux on the resolution of FFE.

3.2. Sample loading control by injection

Sample loading in FFE was an important parameter. It was revealed in Table 1 that the sample loading could be conveniently controlled via the sample flux. If the sample flux was high, the sample loading was high accordingly, and conversely it was low. Thus, the sample loading was under a direct control via the injection system. However, the sample loading could not increase unconditionally. The resolution was a basic factor for our consideration which was relative to the sample flux determining the loading, and the background flux deciding the residence time. Generally, a just complete separation was needed for the purification of target solute(s).

Fortunately, the injection mode made us facile to adjust sample and background fluxes. It could be observed in Table 1 that a better resolution could be achieved if a lower sample loading was used, or if a longer residence time, viz., lower background flux, was used with invariable sample loading. The comparisons of loading datum in Table 1 clearly demonstrated that an approximate maximum sample loading, viz., the 399.2 ml/24 h sample loading, could be selected under the condition of just complete separation of the two dyes. Therefore, it was helpful to get satisfying sample loading under the condition of just complete separation of two adjoining solutes via the ratios of sample and background fluxes.

4. Conclusions

From the experiments above, it could be concluded that: the manipulation of injection system was quite simple and facile; the injection could be significantly used to control sample band width by adjusting the ratios of sample to background fluxes; the injection could be well applied for the separative regulation of two compounds; and the maximum sample loading could be achieved via resolution comparison of various sample fluxes under constant background flux.

Acknowledgements

The project was supplied by the NSFC (Nos. 20675051, 30821005 and 20805031), national 973 Program (No. 2009CB118906), '863' Program (No. 2007AA10Z401) and Instrumental Analytic Centre of SJTU.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.01.085.

References

[1] K. Hannig, Electrophoresis 3 (1982) 235.
[2] L. Krivankova, P. Bocek, Electrophoresis 19 (1998) 1064.
[3] V. Kasicka, Electrophoresis 30 (2009) S40.
[4] A. Tiselius, Trans. Faraday Soc. 33 (1937) 524.
[5] J.St.L. Philpot, Trans. Faraday Soc. 36 (1939) 38.
[6] J. Barrolier, E. Watzke, H. Gibian, Z. Naturforschung. B 13 (1958) 754.
[7] K. Hannig, Z. Anal. Chem. 181 (1961) 244.
[8] A. Kolin, P. Cox, Proc. Natl. Acad. Sci. U.S.A. 52 (1964) 19.
[9] M.J. Clifton, Electrophoresis 14 (1993) 1284.
[10] T.M. Grateful, E.N. Lightfoot, J. Chromatogr. 594 (1992) 341.
[11] C.F. Ivory, J. Chromatogr. 195 (1980) 165.
[12] C.F. Ivory, Electrophoresis 2 (1981) 31.
[13] S. Ostrach, J. Chromatogr. 140 (1977) 187.
[14] P.H. Rhodes, R.S. Snyder, G.O. Roberts, J. Colloid Interf. Sci. 129 (1989) 78.
[15] A. Strikler, T. Sacks, Ann. N.Y. Acad. Sci. 209 (1973) 497.

[16] V. Kasicka, Z. Prusik, P. Sazelova, J. Jiracek, T. Barth, J. Chromatogr. 796 (1998) 211.
[17] V. Kasicka, Z. Prusik, J. Pospisek, J. Chromatogr. 608 (1992) 13.
[18] W. Thormann, R.A. Mosher, Electrophoresis 11 (1990) 292.
[19] W. Thormann, M.A. Firestone, J.E. Sloan, T.D. Long, R.A. Mosher, Electrophoresis 11 (1990) 298.
[20] G. Weber, P. Bocek, Electrophoresis 19 (1996) 1896.
[21] G. Weber, J. Bauer, Electrophoresis 19 (1998) 1104.
[22] M.C. Roman, P.R. Brown, Anal. Chem. 66 (1994) 86A.
[23] K. Hannig, H. Wirth, B.-H. Meyer, K. Zeiller, Hoppe Seyler Z. Physiol. Chem. 356 (1975) 1209.
[24] K. Hanning, H. Wirth, R.K. Schindler, K. Spiegel, Hoppe Seyler Z. Physiol. Chem. 358 (1977) 753.
[25] D.E. Raymond, A. Manz, H.M. Widmer, Anal. Chem. 68 (1996) 2515.
[26] D. Kohlheyer, G.A.J. Besselink, S. Schlautmann, R.B.M. Schasfoort, Lab. Chip 6 (2006) 374.
[27] H. Kobayashi, K. Shimamura, T. Akaida, K. Sakano, N. Tajima, J. Funazaki, et al., J. Chromatogr. A 990 (2003) 169.
[28] C.-X. Zhang, A. Manz, Anal. Chem. 75 (2003) 5759.
[29] B.R. Fonslow, V.H. Barocas, M.T. Bowser, Anal. Chem. 78 (2006) 5369.
[30] B.R. Fonslow, M.T. Bowser, Anal. Chem. 78 (2006) 8236.
[31] D. Kohlheyer, J.C.T. Eijkel, A.V.D. Berg, R.B.M. Schasfoort, Electrophoresis 29 (2008) 977.
[32] V.R. Huebner, R.H. Lawson, Sep. Sci. Technol. 3 (1968) 265.
[33] A. Strickler, Sep. Sci. Technol. 2 (1967) 335.
[34] J. Bauer, G. Weber, J. Disp. Sci. Technol. 19 (1998) 937.
[35] P. Glukhovskij, G. Vigh, Electrophoresis 22 (2001) 2639.
[36] I. Spanik, G. Vigh, J. Chromatogr. A 979 (2002) 123.
[37] S. Chen, J.F. Palmer, W. Zhang, J. Shao, S. Li, L.Y. Fan, R. Sun, Y.C. Dong, C.X. Cao, Electrophoresis 30 (2009) 1998.
[38] C.X. Cao, J. Chromatogr. A 813 (1998) 153.
[39] C.X. Cao, Y.Z. He, M. Li, Y.T. Qian, S.L. Zhou, L. Yang, et al., Anal. Chem. 74 (2002) 4167.
[40] C.X. Cao, L.Y. Fan, W. Zhang, Analyst 133 (2008) 1137.
[41] J. Junkers, P. Schmitt-Kopplin, J.-C. Munch, A. Kettrup, Electrophoresis 23 (2002) 2872.