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Highly-sensitive micellar electrokinetic chromatographic analysis of dioxin-related compounds using on-line concentration

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Abstract

An application study of an on-line concentration technique of neutral analytes for micellar electrokinetic chromatography (MEKC) was carried out in environmental analysis to enhance the UV detection sensitivity. Several dioxins and related compounds, such as dibenzofuran, dibenzo-*p*-dioxin, 2,3- and 2,7-dichlorodibenzo-*p*-dioxins, and 2,3,7-trichlorodibenzo-*p*-dioxin, were used as test solutes. For a highly sensitive separation and detection, cyclodextrin-modified MEKC (CD-MEKC) under acidic conditions was employed as a separation mode and stacking using reverse migrating micelles and a water plug (SRW) as an on-line concentration technique. Almost a 200-fold gain in detection sensitivity was obtained for the model compounds in SRW–CD-MEKC compared to that in normal CD-MEKC without on-line concentration and the limit of detection was found to be around 0.1 ppm for each solute.

Keywords: Sample handling; Dibenzodioxin; Dibenzofuran; Polychlorinated dibenzo-*p*-dioxins

1. Introduction

Since micellar electrokinetic chromatography (MEKC) was developed and introduced into the research field of capillary electrophoresis (CE), the application area of CE has been remarkably broadened as both ionic and neutral analytes can be separated by MEKC [1]. One practical application of MEKC is environmental analysis or separations of environmental pollutants. Capillary gas chromatography (cGC) is, of course, an attractive separation method with high sensitivity and high speed analysis for volatile and thermally stable compounds, while it cannot be applicable to non-volatile or thermally unstable compounds. Since analytes in environmental science contain the latter kinds of compounds, i.e., non-volatile or thermally unstable, MEKC is useful for the analysis of these samples through its high efficiency and sensitivity for a wide range of analytes. Some analytes of environmental pollutants have been separated and detected by MEKC, such as polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins (PCDDs) [2], phthalates [3], anilines [4], pesticides [5].

Recently, PCDDs have become important environmental pollutants as endocrine disrupting chemicals. Currently the analysis of PCDDs in the environment is performed mainly by cGC combined with mass spectrometry (MS), in which high resolution and high sensitivity can be attained. However, the meth-

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od requires a rather large amount of sample and hence, it requires high cost and time consuming pre-treatments of the samples as well as expensive GC–MS instrumentation. By applying MEKC to the analysis of PCDDs, we can expect to reduce the sample amount as well as obtain a simple method that requires less energy and less organic and/or aqueous solvents, in other words, an environmentally friendly method. Previously, a few reports on the analysis of PCDDs have appeared [2,6–8], in which cyclodextrin (CD)-modified MEKC (CD-MEKC) was usually employed because of the high hydrophobicities of PCDDs. In these experiments, sodium dodecyl sulfate (SDS) solutions containing γ-CD and urea were mainly used as separation solutions and, for example, some structural isomers of dichlorodibenzo-*p*-dioxins, trichlorodibenzo-*p*-dioxins, and tetrachlorodibenzo-*p*-dioxins have been successfully separated by CD-MEKC using UV detection. However, detection sensitivity has not been much considered in each case and the sensitivity is not still satisfactory.

The low UV sensitivity is one of major problem in MEKC or CE due to a short path-length through on-column detection, and many efforts have been made to improve the sensitivity. As one of solutions of this problem, we have developed several on-line concentration methods for neutral analytes in MEKC to increase the UV sensitivity by using stacking [9] and sweeping [10] effects. By using these techniques, at least 10-fold and as much as more than 5000-fold concentration efficiencies have been achieved. One of these techniques using stacking effects, stacking using reverse migrating micelles and a water plug (SRW) has been introduced [11], in which an acidic separation solution is used, and it will provide more than 100-fold concentration efficiency. In the present investigation, an application study of the SRW technique to the analysis of PCDDs by CD-MEKC was attempted. Dibenzofuran and four dioxin related compounds were used as the test solutes and performance of SRW–CD-MEKC was compared to that of conventional or normal CD-MEKC.

2. Experimental

2.1. Materials and apparatus

As test solutes, dibenzofuran (DF) and four dioxin-related compounds, such as dibenzo-*p*-dioxin (DD), 2,3-dichlorodibenzo-*p*-dioxin (23-DCDD), 2,7-dichlorodibenzo-*p*-dioxin (27-DCDD), 2,3,7-trichlorodibenzo-*p*-dioxin (237-T3CDD), the chemical structures are shown in Fig. 1, were used. These compounds were first dissolved in a mixed solution of 1,4-dioxane–ethanol (1:1) at an appropriate concentration. For use in SRW–CD-MEKC, the solutions were diluted with low conductivity micellar solutions. SDS was purchased from Nacalai Tesque (Kyoto, Japan), and γ-CD and urea from Wako (Osaka, Japan). All chemicals were highest grade available and used without further purification. Sepa-

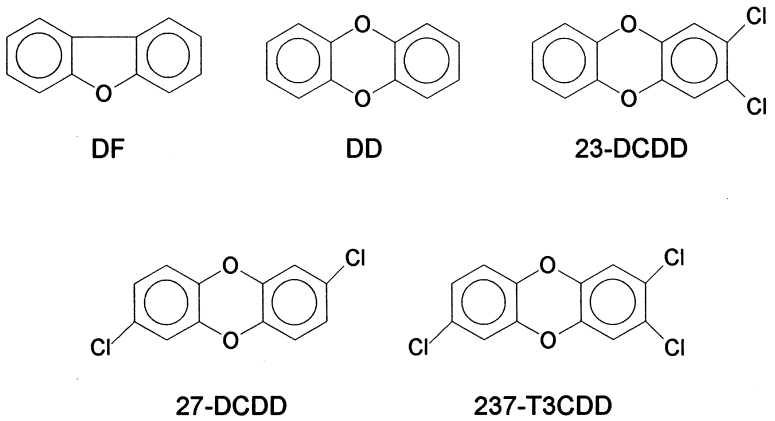

Fig. 1. The chemical structures of dibenzofuran and dioxins used in this work.

ration solutions were prepared by dissolving SDS, γ-CD and urea in 50 m*M* borate or phosphate buffers of pH 9.0 and 2.5, respectively.

For CE, an Otsuka Electronics CAPI-3200Z (Hirakata, Osaka, Japan) instrument equipped with a photodiode array UV detector was used. The separation capillary used was a fused-silica tubing (Polymicro Technologies, Phoenix, AZ, USA) of 40 cm effective length×50 μm I.D.×375 μm O.D. The capillary temperature was maintained at 10°C by an air oven. All injections were carried out hydrodynamically via the height difference between the inlet and outlet vials. In the present experiments, the height difference was fixed as 20 mm and the amount of injection was controlled by the duration of the injection. An applied voltage for separations was kept at 15 kV, but the polarity was changed appropriately.

2.2. Procedure

The on-line concentration technique or SRW was carried out according to the previous report [11], briefly as follows: (1) the separation capillary is filled with a run buffer containing SDS and γ-CD, (2) a long water plug is injected from the injection end followed by the injection of a sample solution, (3) the inlet vial is changed to that of run buffer, then high voltage is applied at negative polarity to effect stacking, removal of the sample matrix and water zone, and separation. The optimum lengths of both the water plug and sample zone were determined by trial-and-error to maximize the enhancement factor [11].

3. Results and discussion

3.1. Normal CD-MEKC

First, the model sample mixture was separated by normal CD-MEKC without on-line concentration under basic conditions according to McClure et al. [8] to examine the performance of CD-MEKC in our system. By using a 100 m*M* SDS–40 m*M* γ-CD containing 5 *M* urea solution (pH 9.0), the five components in the model mixture were successfully separated within a rather long time, 50 min, as shown in Fig. 2a. Here, the concentration of each component was 500 ppm. This concentration is quite high and should be reduced for applying this system to environmental analysis. The separation and detection of a more diluted sample, 40 ppm each, was also attempted under the same conditions, as shown in Fig. 2b. Here, for 27-DCDD (peak 5) the broad peak shape is observed, probably due to a long migration time, and the limit of detection (LOD) for this compound is estimated as low; around 20 ppm from a signal-to-noise ratio (S/N) of 3.

To reduce the analysis time and to use the SRW technique for the separation of the model compounds, acidic conditions were then applied. Under acidic conditions, ca. pH <5.0, the migration direction of the SDS micelle will change from the cathode to the anode [12]. Under these conditions, a solute having a larger retention factor will migrate faster than one having a smaller retention factor. According to the results in Fig. 2, we can expect that 27-DCDD will be detected first and its peak shape be improved due to a reduced migration time. For acidic conditions, an SDS–γ-CD solution of pH 2.5 containing urea was employed. Here, we examined the performance of this separation condition on the test mixture. In Fig. 3, an example of the separation of the test solutes by normal CD-MEKC under acidic conditions without SRW is shown, in which the same peak numbers as those in Fig. 2 are used. Here, the migration order is completely reversed from that in Fig. 2, even the smallest retention factor solute, 23-DCDD, could be detected at the anodic end within the reduced migration time. Peak 5, 27-DCDD, shows an improved shape compared to that in Fig. 2, as mentioned above.

3.2. SRW–CD-MEKC

After the results mentioned above, we expected to adapt the acidic system to SRW–CD-MEKC. The SRW technique will provide a high concentration efficiency to hydrophobic compounds or large retention factor solutes. In SRW–CD-MEKC, a run buffer of low pH must be employed so that the migration velocity of the micelle should be enough large to obtain high efficiency in concentration [11]. The enhancement factor or concentration efficiency depends on the length of the water zone as well as

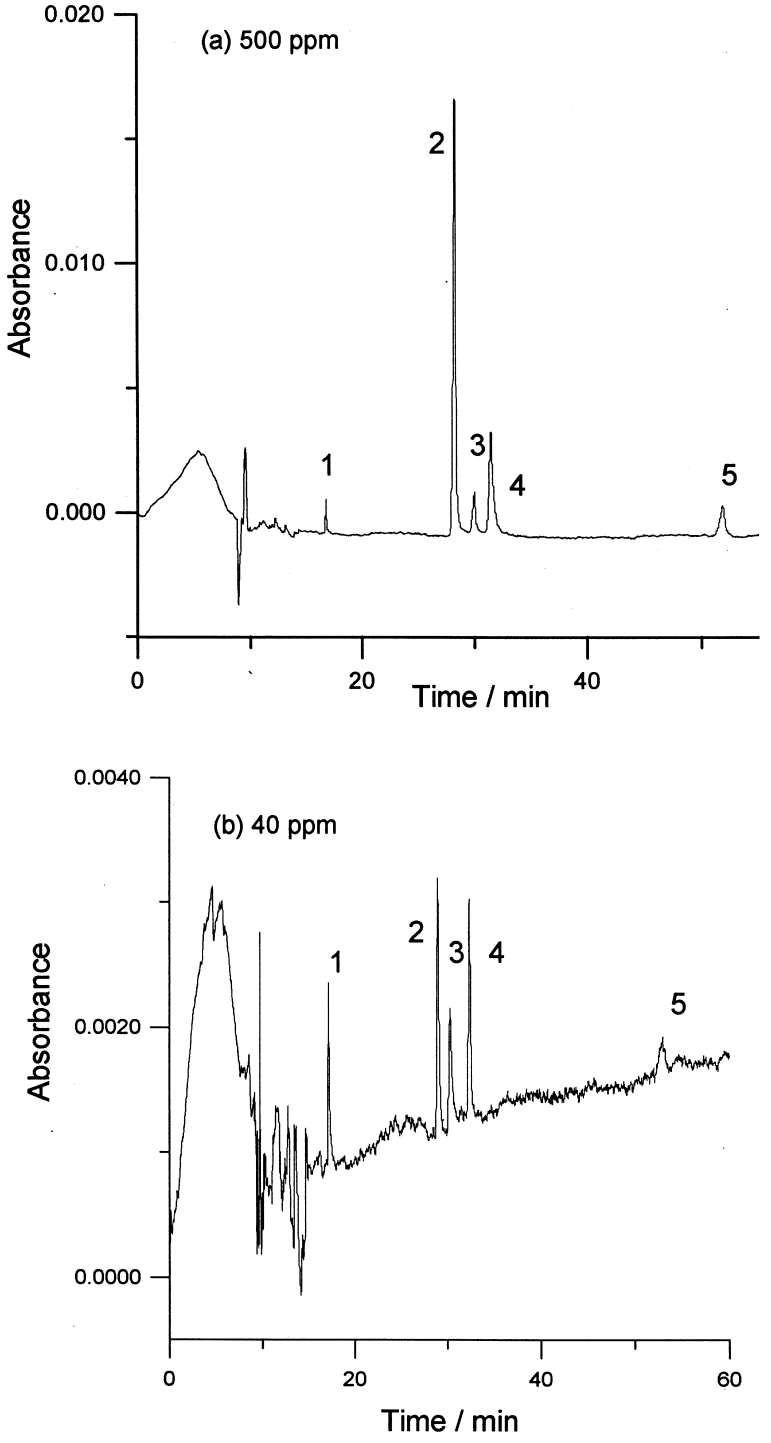

Fig. 2. Separations of the test solutes by normal CD-MEKC under basic conditions: (1) 23-DCDD, (2) DF, (3) 237-T3CDD, (4) DD, (5) 27-DCDD. Concentrations of the solutes, (a) 500 ppm each, (b) 40 ppm each; separation solution, 100 m*M* SDS–40 m*M* γ-CD in 50 m*M* borate buffer (pH 9.0) containing 5 *M* urea; detection wavelength, 225 nm. Other conditions, see text.

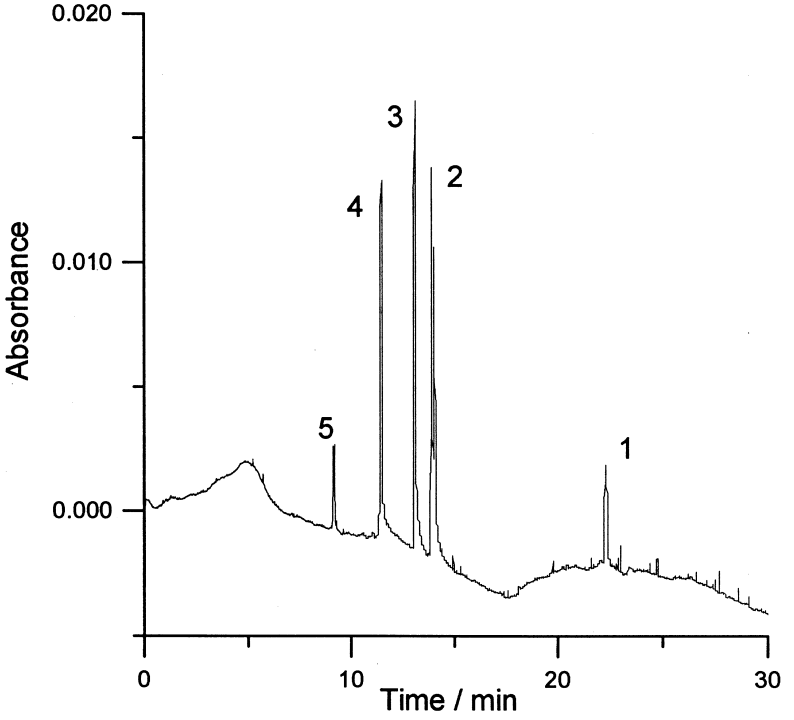

Fig. 3. Separation of the test solutes by normal CD-MEKC under acidic conditions. Solute concentrations, 500 ppm each; separation solution, 100 m*M* SDS–40 m*M* γ-CD in 50 m*M* phosphate buffer (pH 2.5) containing 5 *M* urea. Other conditions as in Fig. 2.

the sample zone [11]. To optimize the lengths of these zones, the durations of the injections were investigated and determined, while the injection height was fixed at 20 mm.

Fig. 4a shows an example separation of the test solutes by SRW–CD-MEKC in which the injecting durations of the water plug and sample zones were both 300 s. Here, the concentration of each solute is 2.5 ppm for DF, DD and 23-DCDD and 5 ppm for 27-DCDD and 237-T3CDD. The UV sensitivity was obviously improved compared to that in normal CD-MEKC. However, decreased resolution between peaks 3 (237-T3CDD) and 4 (DD) was observed. This was affected by both water and sample zones.

Further experiments were carried out concerning the durations of the injections to improve resolution as well as detection sensitivity. As a result, we found that the 500 s injection of the water plug and the 200 s injection of the sample zones brought the maximum enhancement factor as well as good peak shapes and resolution. A typical SRW–CD-MEKC separation of the test mixture is shown in Fig. 4b, where the concentration of each solute is 0.62 ppm in the sample solution injected. At this instance, the estimated value of the LOD is around 0.1 ppm ($S/N=3$) or less. In the SRW technique, a large amount (absolute amount) of analytes can be injected and that is why the concentration sensitivity is increased. In this experiment, actually we are uncertain how much amount (mass) of the analytes were injected. However, in the case that highly diluted solutes should be analyzed while the amount of the sample solutions are enough available, the concentration sensitivity is more important than the mass sensitivity. This situation is often faced in environmental analysis.

The linearity or dependence of the peak area on solute concentration in the SRW–CD-MEKC separation was roughly examined. The relationship between the concentration and peak area for each solute was investigated for three concentration levels, such as 0.31, 0.62 and 1.25 ppm, as shown in

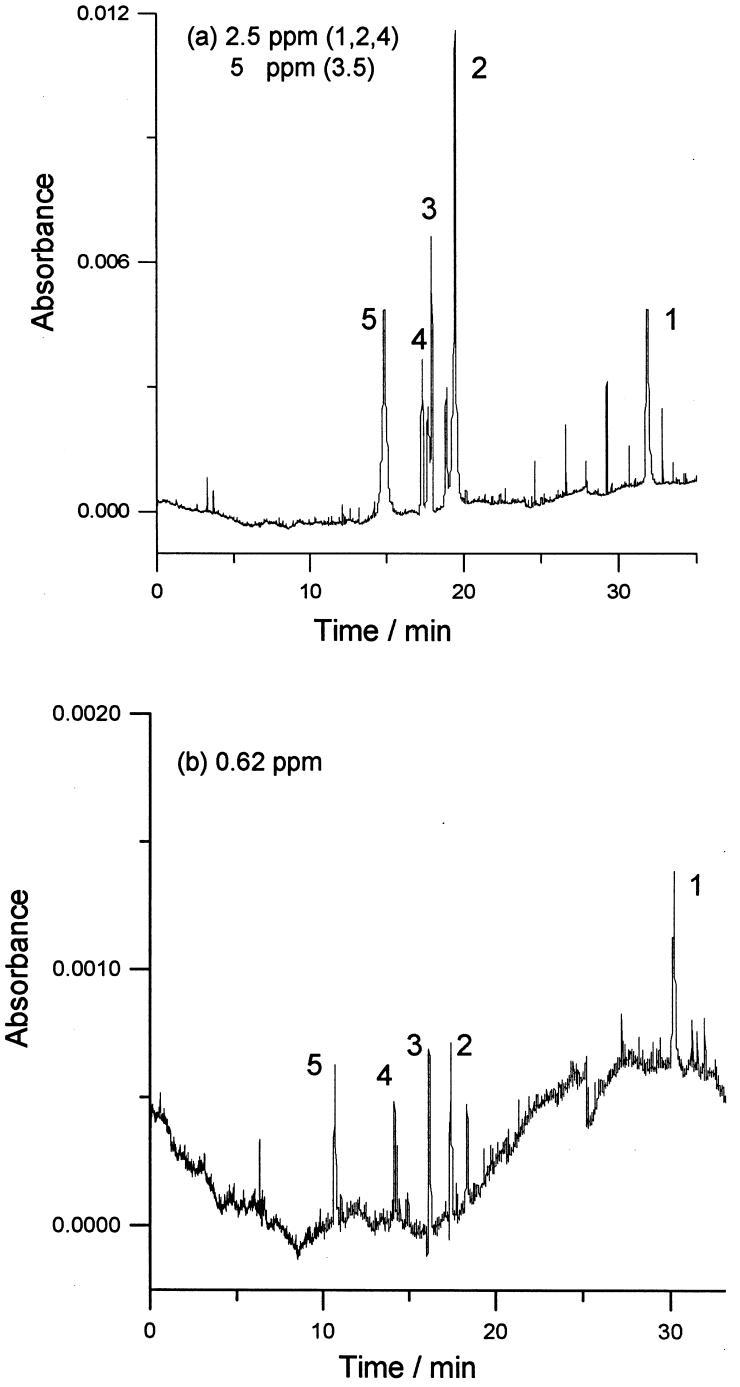

Fig. 4. Separations of the test solutes by SRW–CD-MEKC. Injections, (a) water plug, 300 s; sample, 300 s, (b) water plug, 500 s; sample, 200 s. Solute concentrations, see text. Other conditions as in Fig. 3.

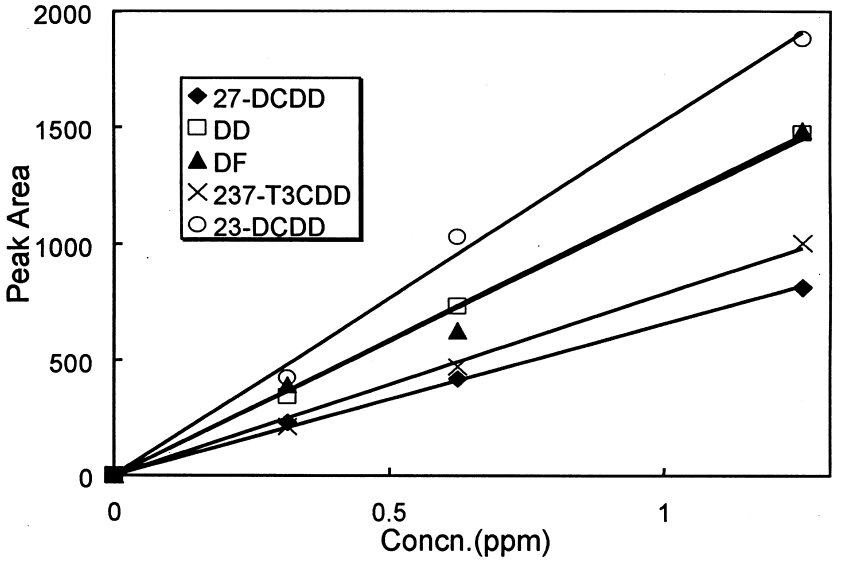

Fig. 5. Dependence of the peak area on the solute concentration in SRW–CD-MEKC.

Fig. 5. Good linearity was obtained and the present SRW–CD-MEKC system would be useful for quantitative analysis.

4. Conclusions

In the normal CD-MEKC system or without any concentration procedures under basic conditions, the estimated value of LOD was around 20 ppm for each solute. By applying the SRW technique, the LOD was successfully decreased to around 0.1 ppm, or 200-fold improvement in detection sensitivity was achieved. Although the present investigation is rather preliminary and the solutes contain only three isomers out of the many PCDDs, the method is expected to be applied to environmental analysis, especially to diluted solutions. As several modes of on-line concentration for neutral analytes in MEKC are now available, improvements of the present method are also in progress by using other concentration techniques.

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References

[1] K. Otsuka, S. Terabe, Bull. Chem. Soc. Jpn. 71 (1998) 2465–2481.
[2] S. Terabe, Y. Miyashita, O. Shibata, E.R. Barnhart, L.R. Alexander, D.G. Patterson, B.L. Karger, K. Hosoya, N. Tanaka, J. Chromatogr. 516 (1990) 23–31.
[3] S. Takeda, S. Wakida, M. Yamane, A. Kawahara, K. Higashi, Anal. Chem. 65 (1993) 2489–2492.
[4] S. Takeda, S. Wakida, M. Yamane, A. Kawahara, K. Higashi, J. Chromatogr. A 653 (1993) 109–114.
[5] S. Wakida, S. Takeda, M. Yamane, A. Kawahara, K. Higashi, Anal. Sci. 7 (1991) 1109–1110.
[6] D.G. Patterson Jr., Z. Liu, J. Grainger, P. McClure, B. Botero, Organohalogen Compd. 19 (1994) 203–208.
[7] J. Grainger, P.C. McClure, Z. Liu, B. Botero, S. Sirimanne, D.G. Patterson Jr., M. Sewer, C. Gillyard, K. Kimata, K. Hosoya, T. Araki, N. Tanaka, Chemosphere 32 (1996) 13–23.

[8] P.C. McClure, J. Grainger, J.R. Barr, D.G. Patterson Jr., Organohalogen Compd. 31 (1997) 187–192.
[9] J.P. Quirino, S. Terabe, J. Cap. Electrophoresis 4 (1997) 233–245.
[10] J.P. Quirino, S. Terabe, Science 282 (1998) 465–468.
[11] J.P. Quirino, K. Otsuka, S. Terabe, J. Chromatogr. B 714 (1998) 29–38.
[12] K. Otsuka, S. Terabe, J. Microcol. Sep. 1 (1989) 150–154.