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Sample stacking using field-amplified sample injection in capillary zone electrophoresis in the analysis of phenolic compounds

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Abstract

This paper discusses a simple on-column concentration with a field-amplified injection technique, using eleven US Environmental Protection Agency (EPA) priority phenols as model compounds. The capillary dimensions (length and internal diameter) for enhancing the on-column enrichment for phenolic compounds are studied. Under the proposed operational conditions, the detection limits ($S/N=3$) were in the ppb range, and the relative standard deviations for the compounds were between 1.4 and 8.8%. Field-amplified injections are preferred to off-line preconcentration methods because of their simplicity, rapidity and high enrichment factors. © 1997 Elsevier Science B.V.

Keywords: Field-amplified injection; Injection methods; Phenols

1. Introduction

Capillary electrophoresis (CE) has become a major separation technique for analysing small volumes of samples [1]. Nevertheless, most of the instruments are provided with a UV absorbance detector and the light path is limited to the inner diameter of the capillary which is, according to Beer's Law, not very attractive for sensitive detection [2]. Recently, fluorescence detection, particularly laser-induced fluorimetry has become popular mainly because of its extremely high sensitivity. Unfortunately, this type of detector is not generally applicable to environmental analysis, because only a few compounds have native fluorescence and most analytes need to derivatize.

An alternative for trace enrichment of samples in CE is on-column sample concentration. Several techniques have been reported for performing on-column concentration to enhance detectability in CE [3].

Isotachophoresis (ITP) can be used as a preconcentration technique for diluted samples in capillary zone electrophoresis (CZE) [4–7]. In this procedure a small quantity of sample is introduced at the interface of a discontinuous buffer system, consisting of a leading (L) and a terminating (T) electrolyte. Although ITP can be used as a preconcentration system, its major disadvantage is that it needs to used several different types of support buffers.

Another system which achieves a high concentration sample in CZE is solid-phase extraction (SPE) inside the capillary. This solid-phase can be incorporated as a short plug at the beginning of the capillary, or as a hydrophobic stationary phase bonded to the inner wall [3]. There are several

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disadvantages of in-column SPE. For example, the sample cannot be introduced directly, because of the risk of blocking the system, and the sample must be cleaned before it is introduced into the capillary.

Field amplified injection techniques have also been used to enhance detectability in CE [8,9]. These techniques are based on the fact that the electrophoretic velocity of an ion depends linearly on the field strength, i.e., the voltage applied divided by the length of the capillary [10]. Before the separation voltage is applied, the sample components are distributed in a relatively broad segment. The electrical resistance of a dilute sample is higher than that of the carrier electrolyte inside the capillary. After the separation voltage has been applied, the electrical field across the sample segment is much higher than across the carrier electrolyte, resulting in a considerably higher velocity for sample ions in the sample segment. When the sample ions reach the boundary electrolyte, they slow down again and stack into a zone which is much shorter than the original sample zone. Thus, the ions are preconcentrated and focused in the column. Chien and Burgi [11] showed that the enrichment factor obtained by field amplified injection can also benefit from the electroosmotic flow (EOF). They used field amplified injection of large sample volumes. In this case a relatively large volume is injected hydrodynamically and the trace enrichment occurs when the polarity is reversed, so pumping out the sample matrix by EOF. Then, the sample continues to be pumped out until the current reaches a value (approx. 95% of its normal value, when no sample zone is present) at which, the polarity is switched back to its original position for the separation step. The main advantage of this system is that without modifying of the instrument, these large-volume injection give enrichment factors of more than 500, thus allowing environmental analysis at low and sub-ppb levels [11].

This paper demonstrates the potential of field-amplified injection by determining eleven US Environmental Protection Agency (EPA) priority phenols, as model compounds, and describes how different capillary lengths and inner diameters enhance the relatively large volume injected hydrodynamically before the use of field-amplified injection in CE. The first experiments were carried out using different sample volumes in a 64.5 cm×75 μm capillary, and the optimum conditions were later adapted to study the effect of different inner diameters and lengths.

2. Experimental

2.1. Instrumentation

Electrophoretic experiments were performed using a Hewlett-Packard (Waldbronn, Germany) Model ^{3D}CE equipped with a diode array detector. Data were collected with the HP Chemstation version A.03.01 chromatographic data system. The separations were carried out using an uncoated fused-silica capillary supplied by Supelco (Bellafonte, USA) and the capillary with an extended light path was supplied by Hewlett-Packard. A detection window was made by burning off the polyimide coating in the capillaries supplied by Supelco. The injection mode was hydrodynamic.

2.2. Chemicals

The eleven phenolic compounds studied were: (1) 2,4-dimethylphenol (2,4-DMP); (2) phenol (Ph); (3) 4-chloro-3-methylphenol (4-C-3-MP); (4) pentachlorophenol (PCP); (5) 2,4,6-trichlorophenol (2,4,6-TCP); (6) 2-methyl-4,6-dinitrophenol (2-M-4,6-DNP); (7) 2,4-dichlorophenol (2,4-DCP); (8) 2-chlorophenol (2-CP); (9) 2,4-dinitrophenol (2,4-DNP); (10) 4-nitrophenol (4-NP); (11) 2-nitrophenol (2-NP). They were all supplied by Aldrich (Beerse, Belgium) except pentachlorophenol, which was obtained from Janssen (Geel, Belgium). A standard solution of 2000 mg l^{-1} of each compound was prepared in methanol and stored in the refrigerator. The working solutions were prepared weekly or daily, depending on their concentration, by diluting the standard solutions with purified water.

Sodium tetraborate (Fluka, Buchs, Switzerland) was used to prepare the electrolyte solution and sodium hydroxide (Aldrich) was used to adjust the pH values of electrolytes and wash capillaries before analysis.

2.3. Electrophoretic conditions and system operation

The electrolyte solution was made by adjusting the pH of a 20 m*M* sodium tetraborate solution to pH 9.9±0.1 with 1 *M* sodium hydroxide. The pH of the electrolyte buffer was checked prior to use, and, if necessary, brought up to pH 9.9±0.1.

At the beginning of each experimental session, the capillary was washed (1000 mbar pressurized flow) with 0.1 *M* NaOH for 15 min followed by a 10 min rinse with water and a 10 min flush with the running buffer, and it was purged for 5 min (1000 mbar) with electrolyte before and after every run. The voltage applied in the separation was 20 kV, and the capillary temperature was kept constant at 35°C. The detector was set at 195 nm. Injection was performed hydrodynamically by a field-amplified system. The length and inner diameter capillary were studied and optimized in this work.

The field amplified preconcentration procedure, according to Nielen [10], involves the following steps: (a) introduction of a very large volume of sample dissolved in water by hydrodynamic injection (50 mbar for 480 s for a capillary of 100 cm×100 μm). (b) Change sample vial for buffer vial and apply a voltage with a reverse polarity (−10 kV outlet positive) to cause the preconcentration of ions (water and positive ions are removed while the capillary retains the negative analytes). When the current reaches approx. 95% of the original value (when capillary is full of electrolyte), the voltage is stopped. This takes 780 s. In this step, careful monitoring of the current is essential to avoid losing the enriched analytes into the buffer vial. (c) Switch back the polarity to its normal position for CZE (20 kV outlet negative). (d) Start CZE separation.

3. Results and discussion

In the present study, CE conditions were adapted from the previously optimized parameters [12,13].

To optimize hydrodynamic injection without removing the sample matrix we used the maximum pressure applied by the instrument (50 mbar). The capillary used was a 64.5 cm×75 μm I.D.. The compounds were dissolved in a water matrix whose conductivity was lower than the supporting electrolyte buffer in the capillary. Figs. 1a–c show the electropherograms recorded for injections of 5, 10 and 15 s, and 1.25, 2.50 and 3.75% of sample length injected, respectively. As can be seen, if the water matrix is not removed from the capillary, the volume of sample which can be injected into the capillary is limited, because when injection time was greater than 10 s, broadened peaks were obtained. This may be due to the laminar broadening mechanism [4] because of the mismatch in the EOFs between the sample plug and the support buffer.

In order to improve detection limits, we studied a preconcentration step using a field-amplified sample stacking system with sample removal [14].

The first experiments were carried out at 50 mbar pressure for 45 s to inject the sample into the 64.5 cm×75 μm I.D. capillary. The sample used was a standard solution of 0.5 mg l^{-1} of each phenolic compound. The main step in these experiments was the time for which the voltage with reverse polarity was applied. Fig. 2 shows that if the current value is lower than 95% (80%) of the current generated when no sample is present, broadened and distorted peaks are obtained, indicating capillary overloading (Fig. 2a), but if it is close to this value (the water plug had been almost completely removed from the capillary; Fig. 2b), the resolution improves. However, if the current value is greater than 95% (98–100%) the signal for the first compounds is lost (Fig. 2c). This could be due to an excessive time that forces these compounds to push out of the capillary, whereas better preconcentration is observed for the rest of compounds. In all three cases the length of the capillary filled was 11.25% (calculated by Poiseuille's law [2]). In this case, we chose 10 kV as voltage with a reverse polarity, because an initial study had shown that values lower than this involved a long preconcentration step, and higher values made it difficult to monitor the current in the final stage of the stacking and led to losses of resolution between peaks.

We have studied different filled lengths of capillary in order to achieve better on-column preconcentration, using the same capillary than before, and the different percentage of the capillary filled was 15, 40 and 96%. We observed that when the percentage of filled capillary increased the peak area also in-

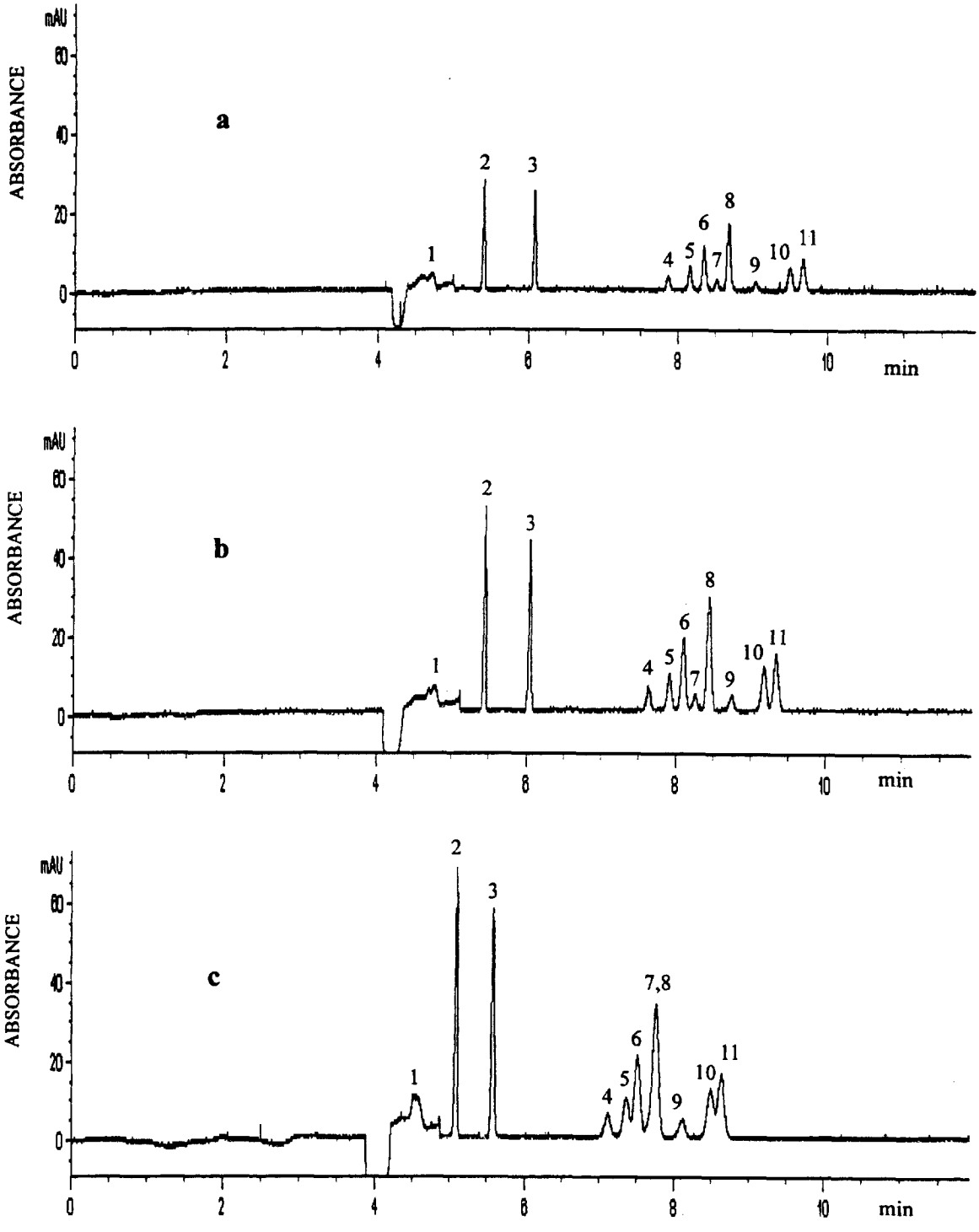

Fig. 1. Electropherograms of 5 mg l^{-1} of each phenolic compound obtained with an injection time of (a) 5 s, (b) 10 s and (c) 15 s. In all experiments the injection pressure was maintained constant at 50 mbar. Capillary: 64.5 cm×75 μm I.D. Electrolyte: sodium borate 20 m*M*, pH 9.9. Separation voltage 20 kV. Detection at 195 nm. For peak identification see Section 2.2.

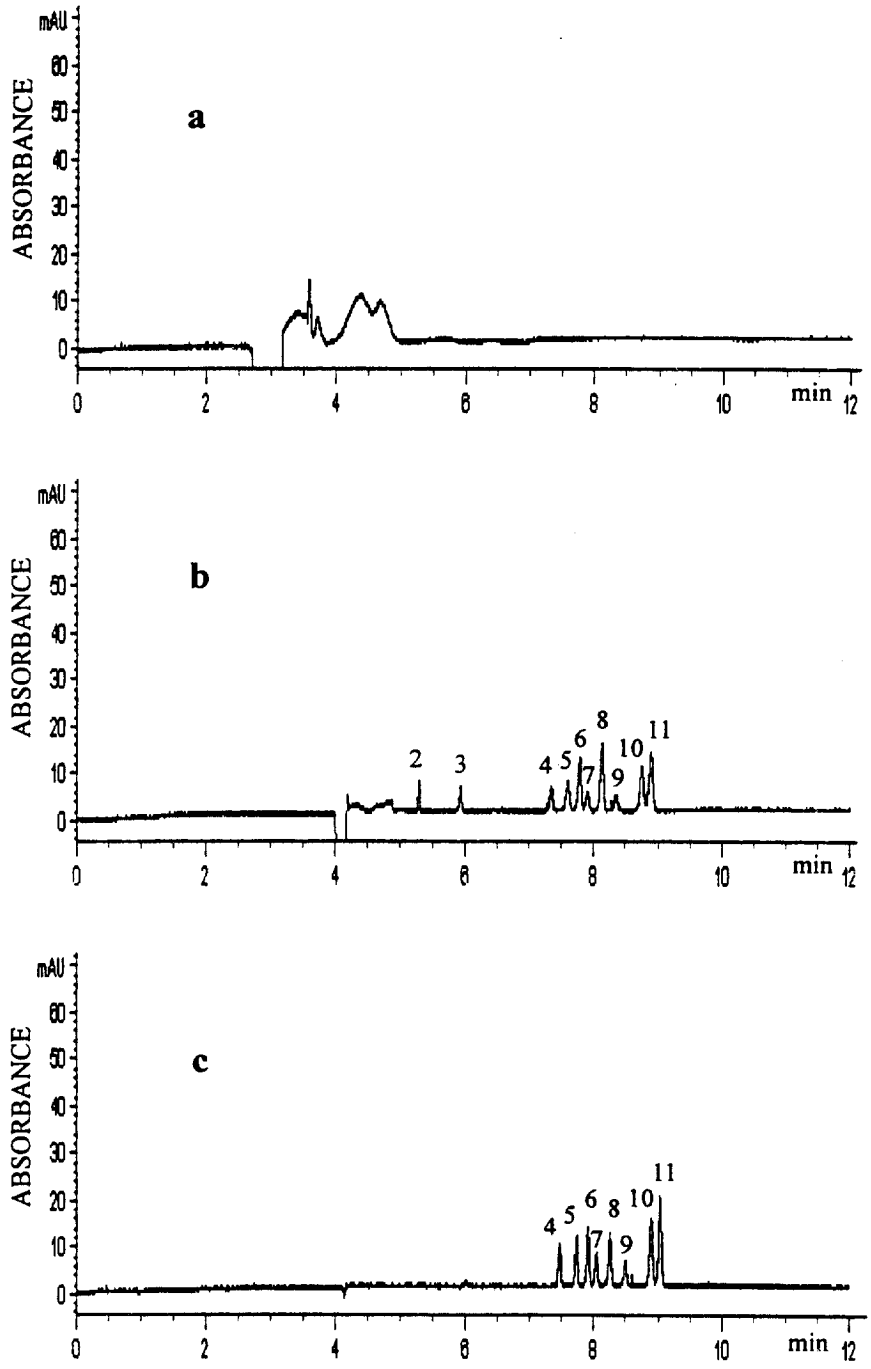

Fig. 2. Electropherograms obtained using a previous preconcentration step with field-amplified sample injection with sample removal. The current value achieved in this preconcentration step was (a) 80%, (b) 95% and (c) 99% when capillary was full of electrolye. For explanation, see Sections 2.3 and 3. Other conditions as in Fig. 1.

creased. The injection length and corrected area (area/migration time) were compared for three phenolic compounds: phenol, 2,4,6-TCP and 2-NP (Fig. 3), and the best results for this injection system were given when the whole of the capillary was filled. These results matched those of Chien and Burgi [14]. However, 2,4-DMP could not be identified at this level of concentration.

In order to increase the amount of analytes injected, we adapted the optimal conditions found with a 64.5 cm×75 μm I.D. capillary to different lengths of capillary: 74 cm, 92 cm and 112 cm. The results are shown in Table 1. It can be seen that the signal increases in all cases, except for 112 cm where for some compounds the signal is lower than in the 64.5 cm capillary. This may be due to an excessively long preconcentration step for reaching of the correct current value. At the beginning, there is no field enhancement because the whole capillary is filled with sample diluted in water, and some of the analytes are carried out of the capillary by the EOF. The best results were found for the 92 cm capillary.

Finally, these conclusions were adapted to capillaries with different internal diameters. Fig. 4 shows the results. For this study, we used a capillary with a "bubble cell" (Fig. 4a), that is, a short section of capillary where the internal diameter has been widened to 150 μm in the detector (normal I.D. is 50 μm), a 75 I.D. capillary (Fig. 4b) and a 100 μm I.D. capillary (Fig. 4c). The total length of the first capillary was 64.5 cm because it was preset by the supplier. The best results were obtained for 100 μm I.D., because the volume of preconcentrated sample is greater than the others.

Table 1
Study of different capillary lengths[a]

Compound	Capillary length (cm)		
	74	92	112
2,4-DMP	–	–	–
Ph	1.19	1.62	0.70
4-C-3-MP	1.13	2.03	0.72
PCP	1.59	2.14	3.58
2,4,6-TCP	1.60	2.22	3.27
2-M-4,6-DNP	1.33	1.79	1.55
2,4-DCP	1.70	2.31	3.60
2-CP	1.16	1.31	0.94
2,4-DNP	1.59	2.11	3.72
4-NP	1.37	1.93	2.76
2-NP	1.48	2.18	2.76

[a] The results are ratios between area obtained for each compound at the different capillary lengths and area obtained using a capillary of 64.5 cm×75 μm I.D.

Fig. 5a,b compares two electropherograms for a 0.5 mg l^{-1} standard of eleven phenolic compounds by both injection systems, hydrodynamic and on-column stacking, respectively. The peak areas obtained by using on-column stacking were between six-times for Ph and twenty-times for 2-CP higher than those obtained by using hydrodynamic injection.

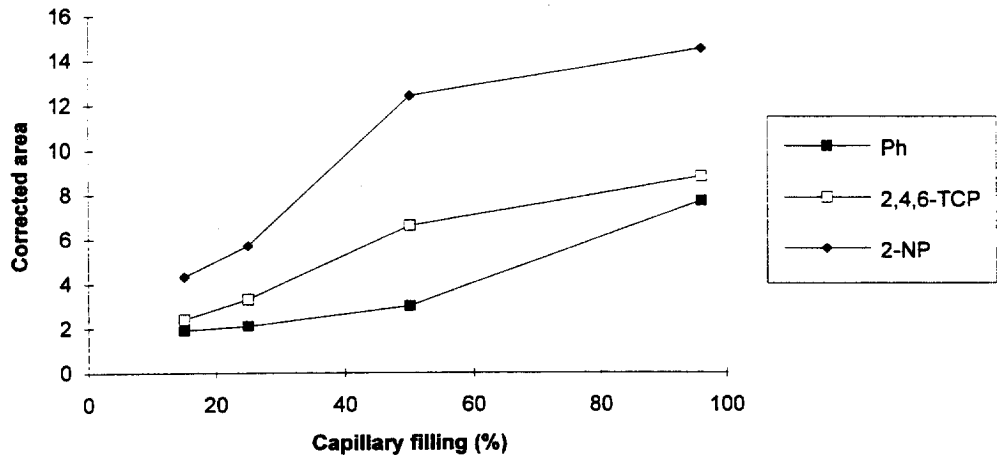

Fig. 3. Study of injection lenght respected to corrected area (area/migration time). For explanation, see Section 3.

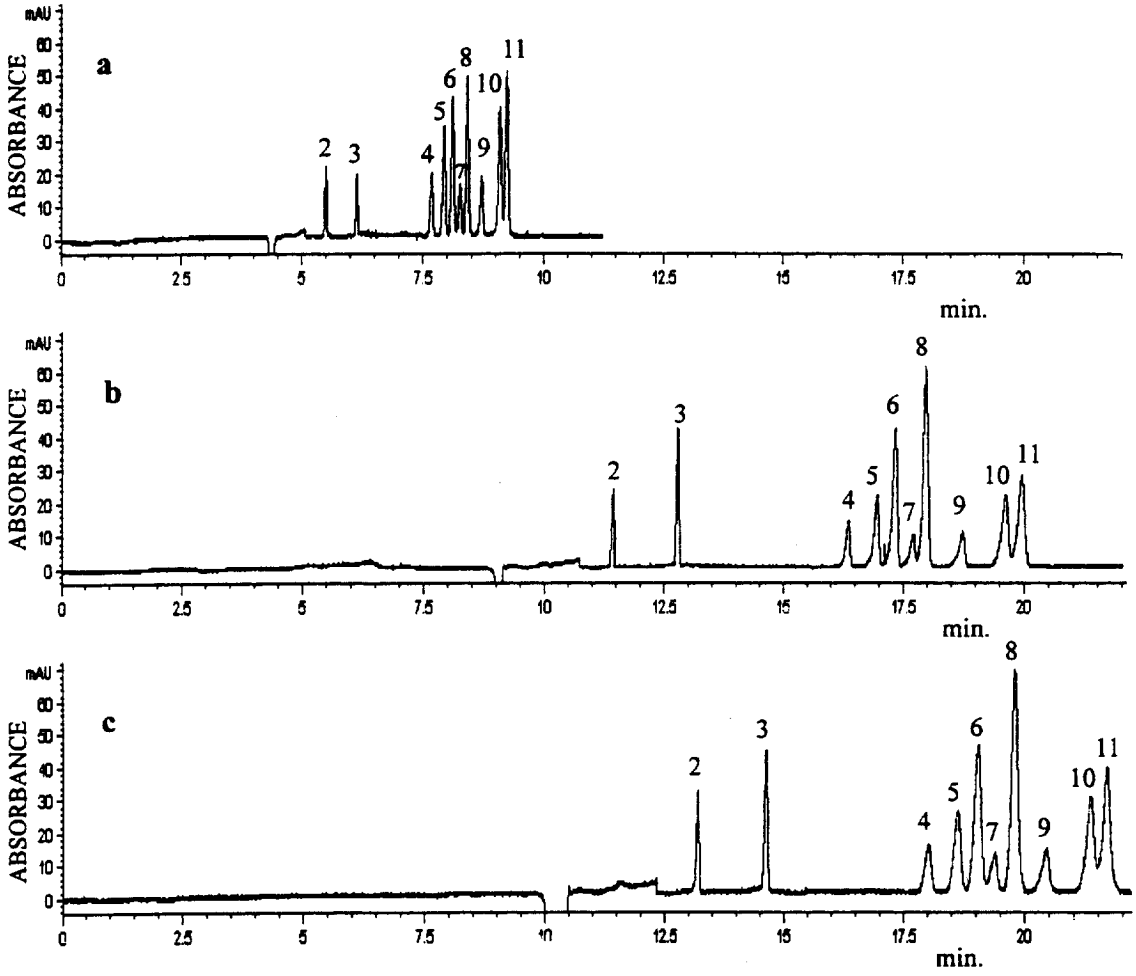

Fig. 4. Electropherograms obtained with (a) a capillary with an extended light path (64.5 cm×50 μm, and 150 μm in the detection zone), (b) a capillary of 75 μm I.D., (c) a capillary of 100 μm I.D. [in (b) and (c) the capillary length is 92 cm]. Other conditions as in Fig. 1.

We studied the linearity of each compound, the correlation (r^2), detection limits and response repeatability in optimal conditions selected. They are shown in Table 2. Within the concentration range studied for each compound, there was a good correlation between peak area and concentration. Repeatability ($n=5$) concentration corresponding to the lowest point on the calibration line for all compounds and the R.S.D.s were between 1.4% for 4-C-3-MP and 8.8% for 2,4-DNP, and the limits of detection (LODs) were calculated using a signal-to-noise ratio criterion equal to 3, and the values obtained were between 0.035 mg l^{-1} for 2-M-4,6-DNP and 2-CP and 0.050 mg l^{-1} for PCP, 2,4-DCP and 2,4-DNP.

4. Conclusions

We have studied a simple on-column concentration technique in an uncoated capillary. If a part of the sample is removed prior to the separation step, large amounts of sample ions can be injected while retaining high-resolution CZE. In an extreme case, we can fill the whole capillary with sample solution which then stacks into a narrow band. When the

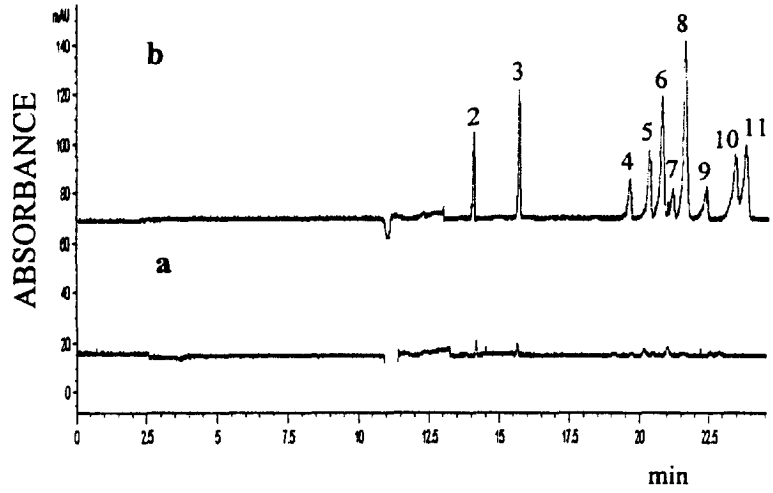

Fig. 5. Electropherograms for the separation of 0.5 mg l^{-1} of each phenolic compound for different injection modes: (a) using hydrodynamic injection (50 mbar, 10 s) without sample removal and (b) using on-column stacking with sample matrix removal (50 mbar, 280 s and then applying −10 kV, 780 s). Capillary of 92 cm×100 μm I.D.. Other conditions as in Fig. 1.

Table 2
pK_a values, calibration data and relative standard deviation for the eleven phenolic compounds

No.	Compound	pK_a	Linear range (mg l^{-1})	Slope	Intercept	r^2	R.S.D (%)[a]
1	2,4-DMP	10.5	–	–	–	–	–
2	Ph	9.9	0.075–2.0	285.2	−7.7	0.9964	7.7
3	4-C-3-MP	9.6	0.075–2.0	412.6	−6.7	0.9969	4.8
4	PCP	4.9	0.100–2.5	299.2	−12.3	0.9984	8.3
5	2,4,6-TCP	7.4	0.075–2.5	541.6	−22.3	0.9985	4.7
6	2-M-4,6-DNP	4.3	0.075–2.0	875.6	−16.5	0.9961	4.4
7	2,4-DCP	7.7	0.100–2.5	244.4	−4.2	0.9989	6.3
8	2-CP	8.1	0.075–2.0	1293.1	−17.8	0.9970	4.2
9	2,4-DNP	4.1	0.100–2.5	316.3	−9.5	0.9986	8.8
10	4-NP	7.2	0.075–2.5	728.8	−27.8	0.9980	2.5
11	2-NP	7.2	0.075–2.5	808.1	−32.3	0.9965	6.7

[a] Obtained for the lowest point on the calibration line ($n=5$) for all phenolic compounds.
Capillary: 92 cm×100 μm I.D.
Injection: see Fig. 5b.

results obtained with this technique are compared with those obtained with a conventional hydrodynamic injection, signals were shown to be enhanced. This type of preconcentation system is less labour intensive, is less time-consuming, and the sample volumes needed are much smaller than off-line preconcentration methods. Finally, we have shown that this technique enables phenolic compounds to be detected in the ppb range, and this enables CE to be used in many environmental analyses.

References

[1] C.A. Monning, R.T. Kennedy, Anal. Chem. 66 (1994) 280R.
[2] R. Kuhn, Capillary Electrophoresis – Principes and Practice, Springer Laboratory, 1993.

[3] D. Barceló, Environmental Analysis, Techiques, Applications and Quality Assurance, (Techniques and Instrumentation in Analytical Chemistry Library, Vol. 13), Elsevier, Amsterdam, 1993.
[4] D. Kaniansky, J. Marák, J. Chromatogr. 498 (1990) 191.
[5] D.S. Stegehuis, H. Irth, U.R. Tjaden, J. Van Der Greef, J. Chromatogr. 538 (1991) 393.
[6] D.S. Stegehuis, H. Irth, U.R. Tjaden, J. Van Der Greef, J. Chromatogr. 591 (1992) 341.
[7] P. Jandik, R. Jones, J. Chromatogr. 546 (1991) 431.
[8] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 141.
[9] R.L. Chien, D.S. Burgi, J. Chromatogr. 559 (1991) 153.
[10] M.W.F. Nielen, Trends Anal. Chem. 12 (1993) 345.
[11] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046.
[12] D. Martínez, E. Pocurull, R.M. Marcé, F. Borrull, M. Calull, J. Chromatogr. A 734 (1996) 387.
[13] D. Martínez, E. Pocurull, R.M. Marcé, F. Borrull, M. Calull, Chromatographia 43 (1996) 619.
[14] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 489A.