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Determination of pentachlorophenol in water samples by capillary zone electrophoresis

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Abstract

Different alternatives are presented for the monitoring of pentachlorophenol (PCP) in water samples by capillary zone electrophoresis. Detection and quantification limits can be adjusted to the required levels by means of sample stacking techniques (with or without sample matrix removal) which, together with the use of extended light path capillaries, allow the determination of PCP in the ppt range required by environmental and toxicological international regulations.

Keywords: Capillary electrophoresis; Water analysis; Sample preparation; Environmental analysis

1. Introduction

In recent years, capillary zone electrophoresis (CZE) has become one of the most outstanding separation techniques in the analysis of an increasingly large number of charged species [1–3]. Characterized by high efficiency and a short run time, its main disadvantage follows from the balance between the sample bandwidth, the total length of the capillary and the diffusion of the analytes during the run. In order to attain high resolution, the analyte bandwidth has to be smaller than those resulting from diffusion and/or the detection window length. Usually this bandwidth is of the order of 1 mm, which implies injection volumes in the range 2–10 nl (for capillaries of 50 μm I.D.). Using the most common on-column UV detection, the detection limits (10^{-6} *M* [4]) are below those needed for many environmental analyses [5].

Two main alternatives can be used to improve CZE–UV detection limits: on-column sample preconcentration [6] and extended light path detection cells [7]. Obviously, off-line preconcentration procedures help further to reach low detection limits where the above-mentioned procedures do not allow the required sensitivity. On-column preconcentration involves several sample stacking techniques [6,8–10], the simplest of which consist in the injection of large volumes of sample dissolved in a lower conductivity buffer matrix than those used for CE separation. Because the resistivity of the sample zone is greater, an increased electric field affects charged species in the sample zone which, under influence of this field, rapidly migrate towards the border region separating the sample and buffer solutions. When charged species reach this

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border region they are restrained by the action of a small electric field, thus focusing in a sharp sample band. Obviously, the stacking mechanism affects both positively and negatively charged species. In an untreated silica capillary under normal electrophoretic conditions (anodic injection), positively charged species are focused at the front of the sample matrix band, whereas negatively charged species are focused at the opposite extreme.

If the sample band becomes too large, hydrostatic pressure can develop because of the differences in local electroosmotic flows on the two sides of the sample–buffer border junction. Moreover, a backwards laminar flow could arise which would lead to band spreading and, consequently, poorer resolution between analyte peaks. In practice, these effects limit the maximum enrichment factor attainable by this sample stacking technique to a factor of about 10 [11]. However, this factor can be increased by taking advantage of the electroosmotic flow (EOF) [9,12–15], which allows one to eliminate the sample matrix during or after the stacking stage, but before the CZE separation, avoiding local EOF differences. The most usual procedure to do this involves field polarity reversion when the capillary has been filled with the sample matrix. Under these conditions, EOF flowing to the injection electrode pulls the sample matrix out of the capillary while negatively charged species under the influence of a high electric field move to the detection electrode, focusing as in other stacking techniques in the border region separating the sample matrix and the separation support buffer. When the current reaches about 95% of its normal value (the experimental value under normal conditions of separation with the considered support buffer in the absence of stacking), the field polarity has to be switched to its normal position. Under these conditions, a sharp focused sample band close to the injection capillary end will undergo the usual CZE separation. This technique, named field amplified injection with matrix removal [16], allows concentration factors higher than 100, enabling the CZE technique to be used in many environmental analyses.

Pentachlorophenol (PCP) is a compound of high environmental concern because of its proved toxicity and wide fields of application [17]. This compound (and also other chlorophenols) has been included in the water and waste water priority pollutant lists [5,18]. Gas chromatography [19] is the usual choice for PCP monitoring. However, its low vapour pressure and acidity imply poor chromatography characteristics or the need for derivatization processes [20]. HPLC [21] and CZE could be used, using the above-mentioned stacking techniques, for the monitoring PCP in waste waters, but for drinking waters the detection limits are far below those attainable by these techniques. A detection limit of 6 ppb ($S/N = 3$) has been reported for PCP by using HPLC with electrochemical detection [22]. However, instability of detector response due to contamination of the electrode by several analytes and their reaction products occurs, leading to a need for frequent cleaning, electrode polishing and passivation, limiting its practical usefulness in the analysis of drinking and natural water samples. In this paper, a procedure is presented that allows the determination of PCP in aqueous samples using field amplification injection with matrix removal together with an extended light path detection window (bubble capillary). This method could be applied to the direct analysis of water samples containing PCP at concentrations greater than 2 ppb, useful for many environmental studies. A modified procedure involving an off-line graphitized carbon solid-phase extraction (SPE) [23,24] is also presented that can be applied to the analysis of drinking waters reaching quantification limits of 60 ppt by processing sample volumes not larger than 100 ml.

2. Experimental

2.1. Apparatus

All the experiments were carried out on an HP^{3D}CE capillary electrophoresis system (Hewlett-Packard, Palo Alto, CA, USA) fitted with an on-column diode-array detector. Spectra were acquired in the range 200–400 nm using 230 nm

for signal monitoring and 450 and 480 nm as references.

Polymide-coated fused-silica capillaries of 56 cm effective length and 50 μm I.D. were prepared from a 25-m capillary supplied by Composite (Teknokroma, Barcelona, Spain). On-column detection windows were made by burning a small section (0.5 cm) of the external polymide coating and removing the burned residue with methanol. HP-CE extended light path capillaries (56 cm × 50 μm I.D.) (Hewlett-Packard) were also used. The support buffer was 40 m*M* sodium borate (pH 10, adjusted with sodium hydroxide). The capillary and vial-tray temperatures were maintained at 20°C, the former by means of the built-in thermostating facility and the latter with the aid of a cooling recirculating bath (RB-5A; Techne, Cambridge, UK). The voltage during separations was 30 kV. Samples were injected by pressure (50 mbar), varying the injection time as a function of the preconcentration procedure used. Under the proposed operating conditions, the analysis time was 13 min.

2.2. Reagents

All standards and buffers were prepared in ultra-pure water obtained from a Milli-Q system (Millipore, Milford, MA, USA). All the solvents and reagents were of analytical-reagent grade supplied by Merck (Darmstadt, Germany). Pentachlorophenol standard was obtained from Aldrich (Milwaukee, WI, USA). A stock standard solution of 4 mg/ml was prepared in methanol and stored in the dark at 4°C while not in use. Working standard solutions of different concentrations were prepared from this stock solution by appropriate dilution with water.

Tap and ultra-pure water samples were preconcentrated using 0.25-g Supelclean Envi-Carb graphitized carbon black SPE cartridges (Supelco, Bellefonte, PA, USA).

2.3. Sample preparation

Samples with or without addition of PCP at concentration levels greater than 2 ppb were analysed as such. More dilute samples were concentrated off-line according to the following procedure. Carbon cartridges were conditioned by passing 10 ml of methanol–pentane–acetone (1:1:1) (this solution ensures cleaning of the cartridge) followed by 10 ml of methylene chloride–methanol (90:10). The cartridge was then activated with 5 ml of ultra-pure water acidified to pH 2 with hydrochloric acid. A 100-ml water sample (spiked or not) was slowly passed through the carbon cartridge with the aid of a vacuum pump or simply by means of a suitable syringe. After the sample had completely passed, the cartridge was rinsed with 5 ml of ultra-pure water and dried for 10 min with the aid of dry nitrogen. Elution of PCP was then accomplished by passing through the cartridge 3 ml of methylene chloride–10 m*M* methanol (90:10) in tetramethylammonium hydroxide (TMAOH). Elution was performed in the opposite direction to that used for sample loading in order to ensure the quantitative recovery of PCP in a small volume of eluate. The eluted PCP was then back-extracted with 3 ml of ultra-pure water (3 × 1 ml). This back-extraction was quantitative and the pH of the final aqueous extract should be ca. 10. The aqueous extract was then ready to be injected and sample stacked in the CE equipment. In this case, samples should contain small amounts of TMAOH.

3. Results and discussion

3.1. Sample stacking without matrix removal

First, experiments were conducted to develop a separation scheme enabling the good resolution of the PCP peak from other chlorophenols that can be found in samples and can be partially recovered in the SPE process under the given conditions. Eight chlorophenols including mono-, di-, tri- and tetrachlorinated phenols were used as potential interferents. In the electropherogram depicted in Fig. 1 it can be clearly appreciated that the PCP peak can be perfectly resolved from all these interferents. This electropherogram was obtained using the operating conditions given in the Experimental section (40 m*M* borate buffer at pH 10 and 20°C).

Once selectivity was assured, a series of ex-

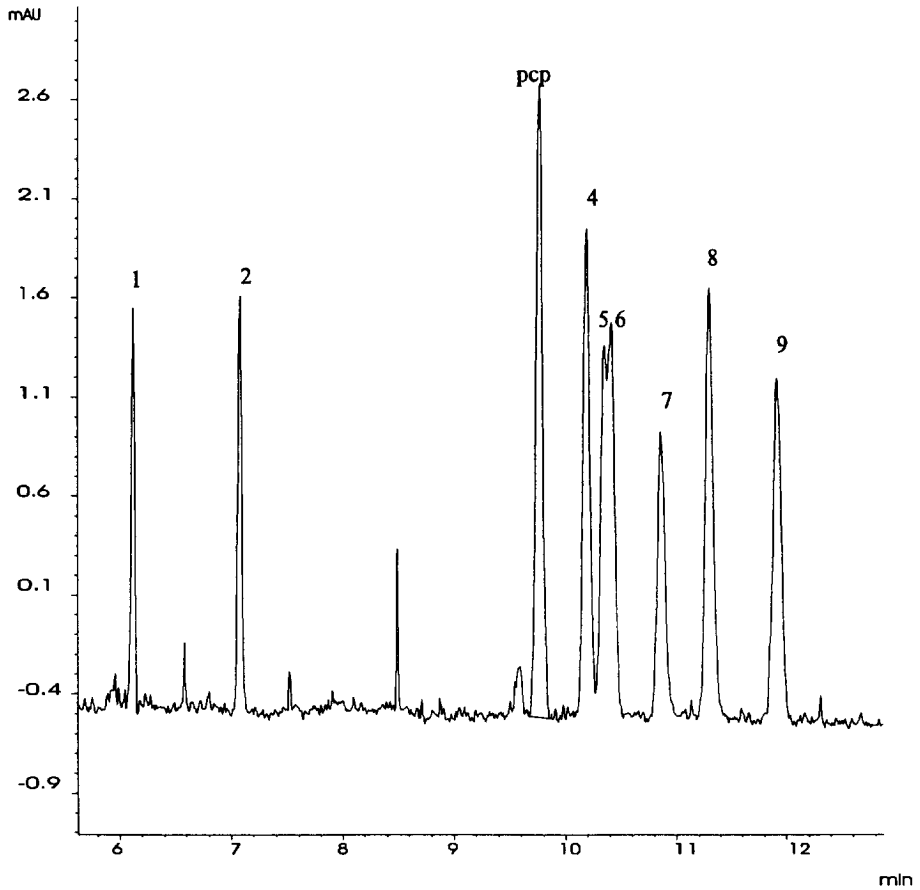

Fig. 1. Electropherogram of a standard mixture of PCP and several chlorophenols. Peaks: 1 = phenol; 2 = 4-chloro-3-methylphenol; 3 = pentachlorophenol (PCP); 4 = 2,3,5,6-tetrachlorophenol; 5 = 2,4,6-trichlorophenol; 6 = 2,4,5-trichlorophenol; 7 = 2,4-dichlorophenol; 8 = 2,3,6-trichlorophenol; 9 = 2-chlorophenol.

periments were conducted to establish the minimum PCP concentration in samples that can be directly determined (without sample stacking). Using a capillary without an extended light path the limit of quantification was about 2 ppm. To establish the quantification limit under conditions of direct sample stacking (without matrix removal), a standard of PCP having a concentration of 4 ppm was injected with a series of increasingly greater injection times from 5 to 40 s (the injection pressure was maintained constant at 50 mbar in all the experiments). Fig. 2 shows the electropherograms obtained for injections of 5, 10, 30 and 40 s. In these experiments, chlorophenols used as model interferents were also added to the PCP sample to evaluate not only the increased response with regard to PCP but also the resolution of the PCP peak from the other possible chlorophenols. As can be seen in Fig. 2, the selectivity for PCP is acceptable even with a 40-s injection time. However, when the injection time was greater than 30 s, a broadened and distorted PCP peak was obtained, indicating capillary overloading. Comparison of the electropherograms obtained with 5- and 30-s injection times shows a concentration factor of about 10, which is in accord with published data for other types of compounds [11]. Thus, using sample stacking without matrix removal, the quantification limit for PCP was established as

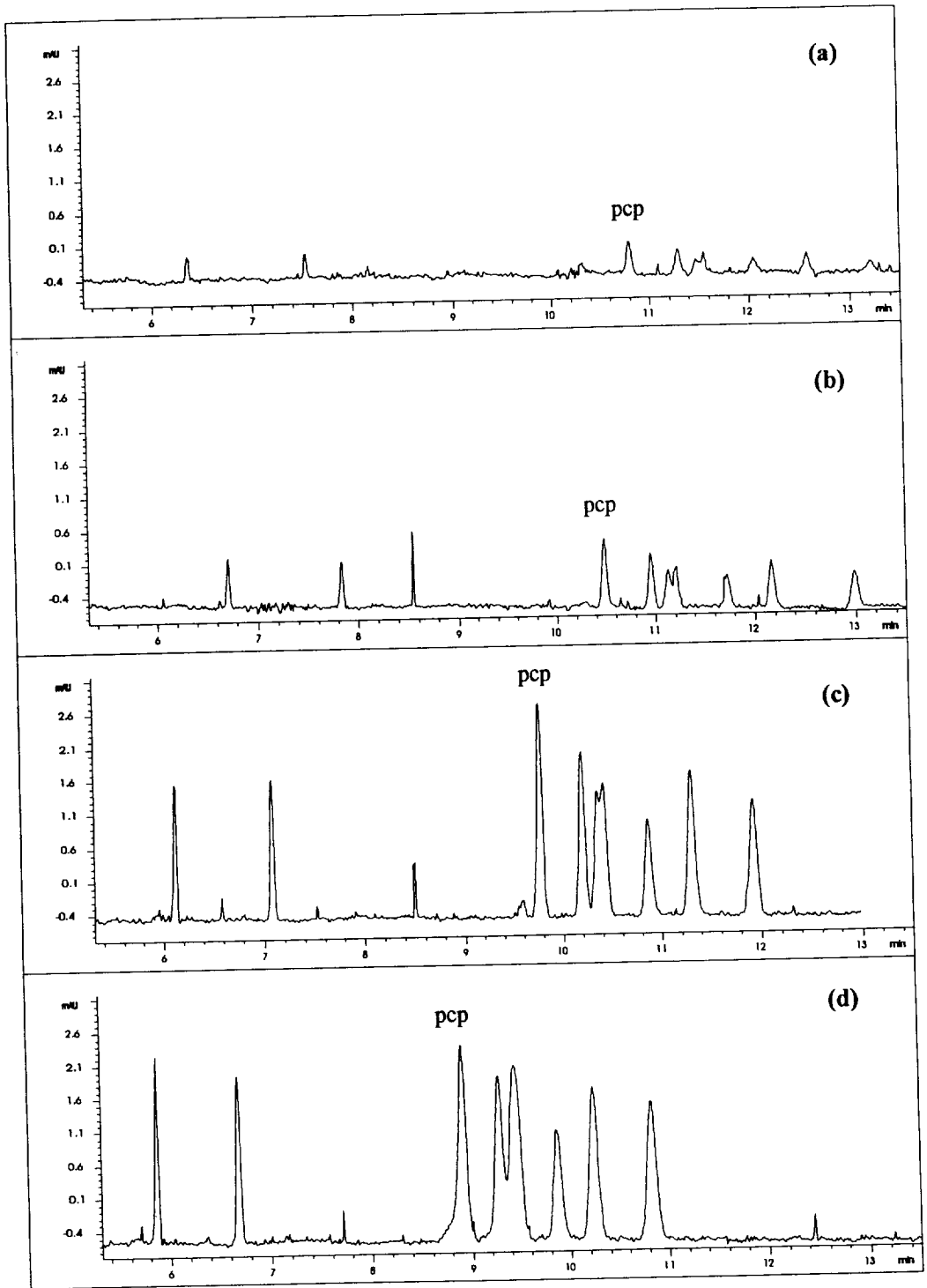

Fig. 2. Electropherogram of a 4 ppm chlorophenol mixture obtained with injection times of (a) 5, (b) 10, (c) 30 and (d) 40 s. In all experiments the injection pressure was maintained constant at 50 mbar.

200 ppb, which is suitable for many environmental and waste water monitoring studies and with the advantages that the analysis is made directly on the water sample, without any prior treatment or manipulation, and the analysis run time compares favourably with those in HPLC and GC procedures.

3.2. Field-amplified sample stacking with matrix removal

As has been demonstrated [9], matrix removal can be used to increase the amount of sample injected in CZE without losing resolution between analytes. In this case, the capillary was filled with very large volumes of sample by applying pressure for several minutes. Once the capillary was filled, the field polarity was switched to −30 kV and the current was monitored. When the current value approximated 95% of the usual value under separation conditions (about 100 μA), the field polarity was changed again to its normal value (30 kV). Under these conditions the sample matrix could be removed from the capillary, ensuring that PCP was not backflushed to the injection buffer reservoir. It was observed that the time needed to complete this operation of sample stacking was very reproducible (0.9 min), providing a consistent sample matrix. Thus, the complete procedure can be automated. As the time needed for matrix backflushing depends on the relative conductances of the support buffer and the sample matrix, the operation can be automated also for different sample matrices by means of a macro associated with the HP-CE instrument which monitors the current and switches the polarity when the current falls above a predefined value (which in turn will depend on the Experimental value obtained in the normal separation of PCP with the support buffer selected).

Fig. 3 shows the variation of the peak area for PCP as a function of the injection time under conditions of sample stacking with matrix removal. It is evident that for injection times greater than 7 min no further increases in the peak area can be obtained. In fact, for injections longer than 8 min a significant triangular peak shape was observed owing to capillary overloading. For

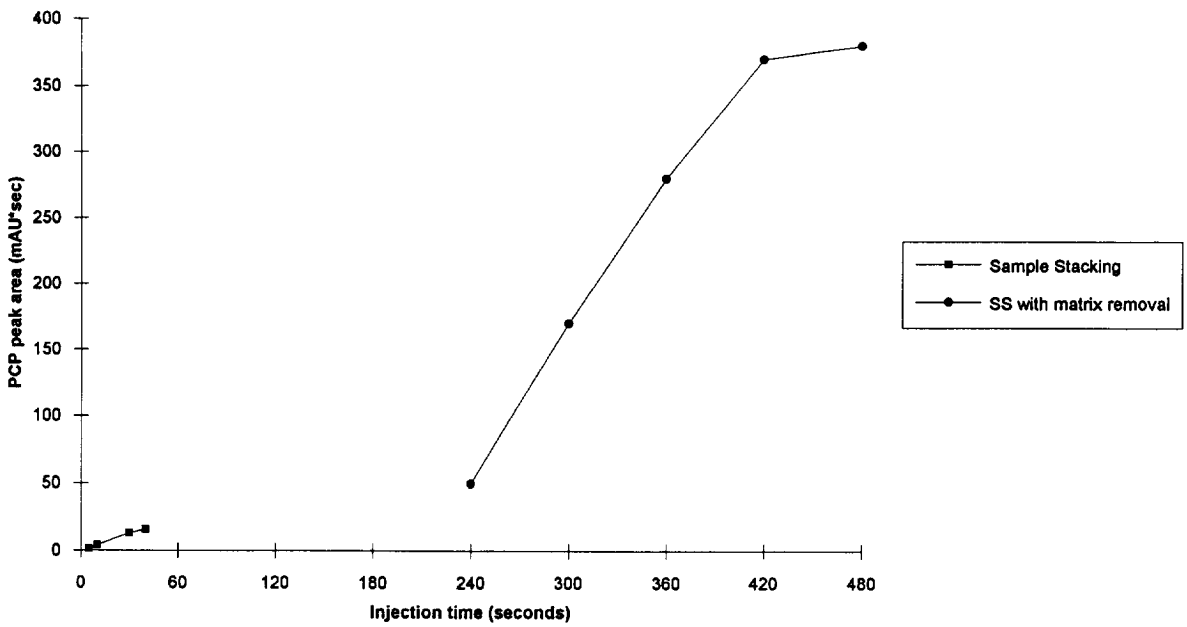

Fig. 3. Variation of the peak area of PCP as a function of the injection time under conditions of sample stacking with and without matrix removal.

comparative purposes, in Fig. 3 the peak area data obtained for injections without matrix removal have also been included. It is clear that matrix removal allows a concentration factor of about 200 compared with the conventional injection (no sample stacking) and a factor of 20 compared with sample stacking without matrix removal. Therefore, the quantification limits attained by this procedure lie in the lower ppb range.

3.3. Use of extended light path capillaries

Up to this point, all the experiments were conducted using conventional straight capillaries with detection windows made manually in the laboratory. In this type of capillary, on-column UV detection is limited by the optical path length, which cannot be greater than the inner diameter of the capillary. The commercially available extended light path capillaries allow a further increase in sensitivity [7]. As the bubble in the capillary is situated only in the detection window, no increase in current appears. Moreover, flow diminishes in the bubble, and the analyte bands expand radially, thus contracting axially. Consequently, the analyte band is not diluted. A decreased flow and extended light path allow better sensitivity by a factor of usually 3–5 [7]. In Fig. 4 the electropherograms obtained for a sample of ultra-pure water spiked with 16 ppb of PCP using conventional and extended light path capillaries are shown. In the former case the peak area for PCP was 1.4 mAU s and in the latter 4.0 mAU s. Thus, a concentration factor of 3 can be obtained by the use of these capillaries, without losing resolution or efficiency.

3.4. Validation of the procedure

Calibration graphs were constructed in the range 5–40 ppb and showed good linearity (correlation coefficient 0.9997, residual standard deviation 0.0396, slope 0.2279 and intercept −0.2692).

The quantification limit calculated for a signal-to-noise ratio of 10 was 1.9 ppb. Reproducibility was assessed by injection of the 5 ppb standard during several days (three injections per day). The relative standard deviation was 7.2%. The migration time repeatability for PCP was 1.3%. The use of a CE system with diode-array detection permits the easy control of peak purity and confirmation of the identity of the PCP peak.

3.5. Analysis of tap water samples

The total content of phenols in drinking waters is limited to 0.5 ppb by most national and international regulations [5,18]. Hence this is also the limit for any individual phenol derivative included in priority pollutant lists, although lower concentrations of individual species could be expected. Clearly, the quantification limit for the proposed procedure does not allow the direct monitoring of PCP in drinking waters. Off-line preconcentration procedures can be used to decrease this quantification limit by one or two orders of magnitude.

The modified procedure involves the preconcentration of 100 ml of water by means of graphitized carbon black (GCB) cartridges to a final volume of 3 ml. With this process a quantification limit of 60 ppt was obtained, which is suitable for drinking water monitoring. Previous studies [25] have shown that even 1500 ml of water samples can be preconcentrated on these cartridges without significant losses. Hence the method can be extended to very low detection limits by increasing the sample volume processed. However, the analysis of relatively small volumes of sample has many advantages. The time needed for pouring the sample through the cartridge decreases dramatically (10 min should be sufficient to pass 100 ml of sample whereas 1500 ml takes about 2 h) and also the manipulation is simplified (100 ml can be handled with a syringe, while greater volumes need special vacuum manifolds).

In previous studies [25], an acidified organic solvent mixture was proposed for GCB cartridge elution. However, in the present work, an alkalinized organic solvent mixture was preferred, for three main reasons: the need to change to an

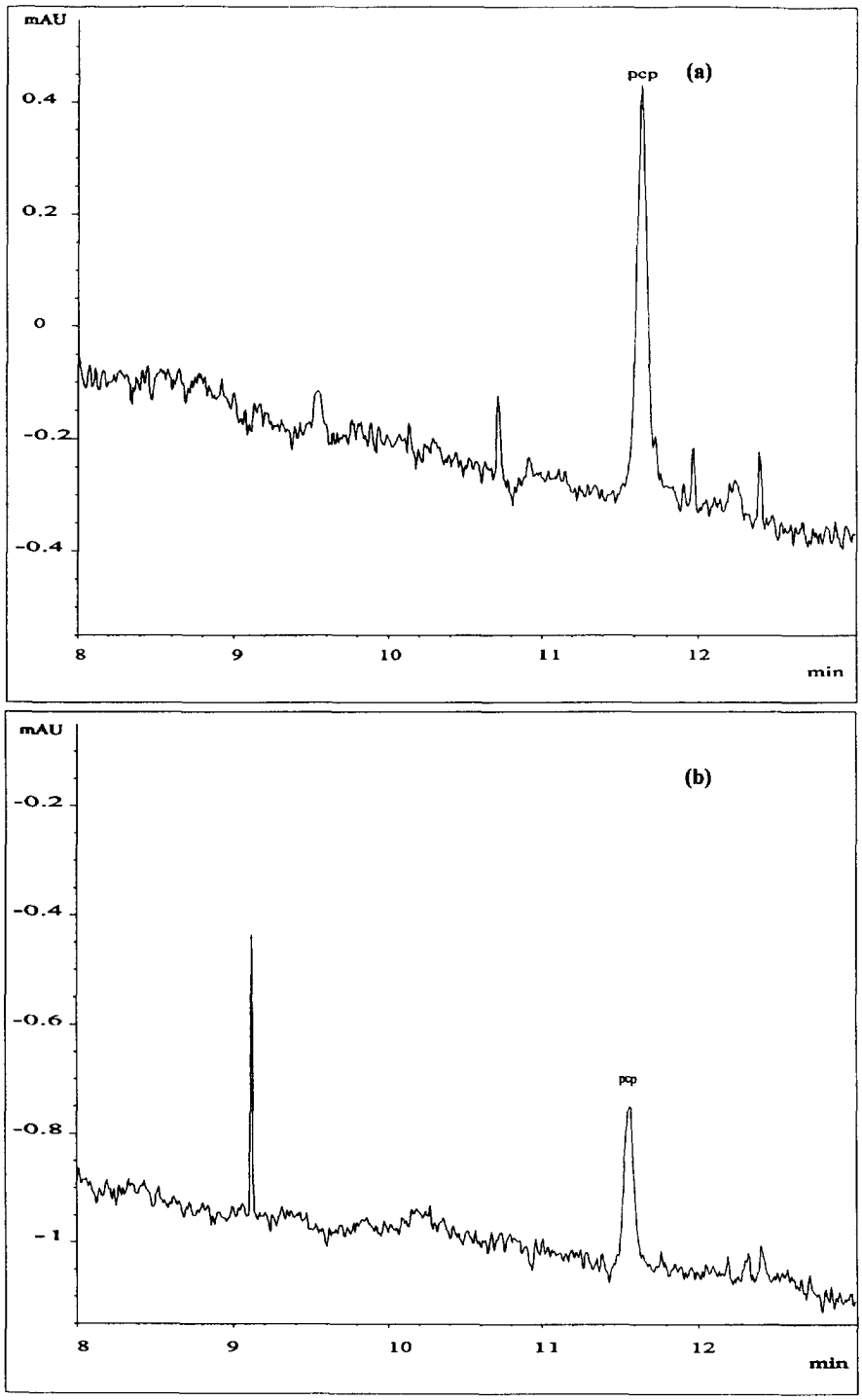

Fig. 4. Electropherogram of a standard solution of 16 μg/l of pentachlorophenol obtained with (a) an extended light path capillary and (b) a conventional straight capillary.

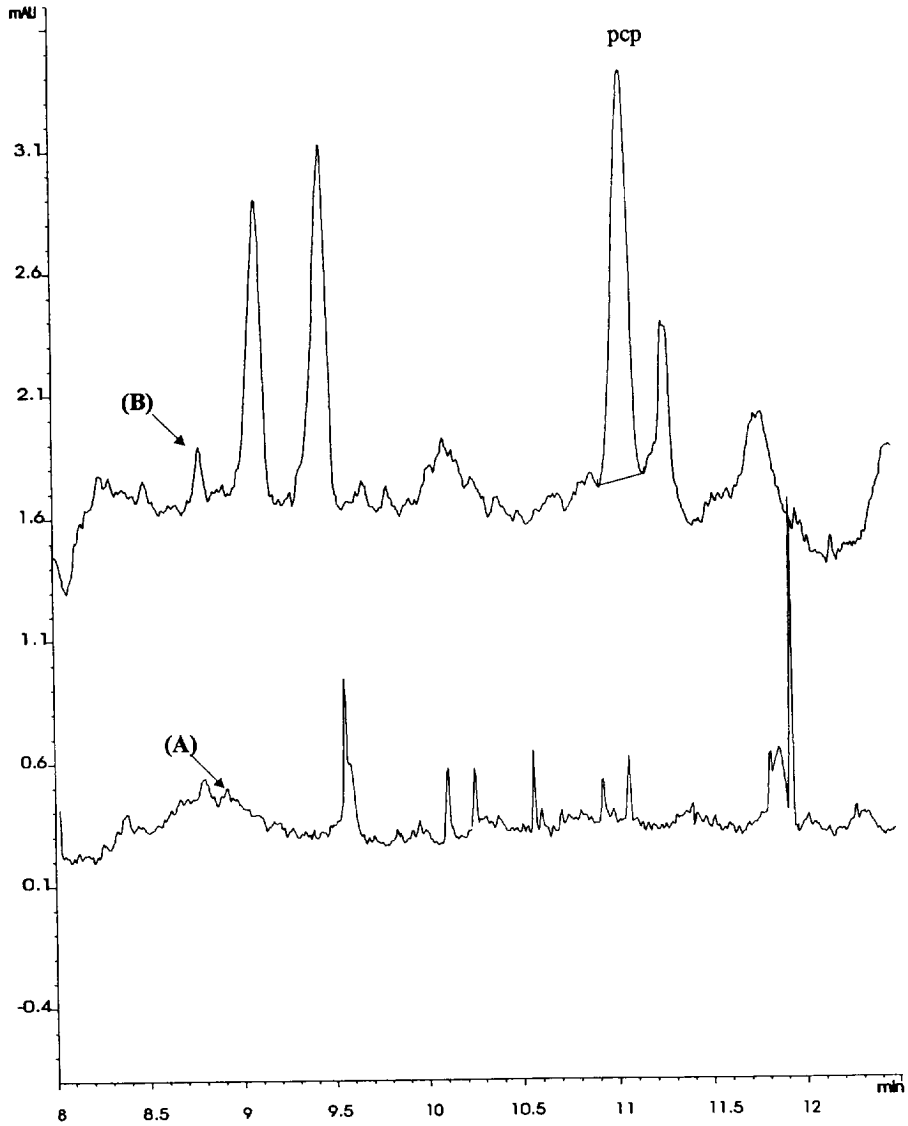

Fig. 5. Electropherogram corresponding to (a) 100 ml of tap water sample and (b) 100 ml of tap water sample spiked with PCP at the 0.5 ppb level.

aqueous solvent the samples before CZE injection, the need to have the injected sample in a matrix exhibiting a significantly lower conductance than the support buffer and the desirability to reduce the sample manipulation to the minimum. Thus, eluting PCP with basified organic mixture allows the transfer of PCP to ultra-pure water simply by back-extraction, without the need for additional reagents or pH adjustment. It was checked that the elution of PCP from the cartridge and back-extraction to water were quantitative under the conditions given under Experimental (the recovery was $95.8 \pm 2.7\%$ for a series of five replicates using a spiked sample at the 0.5 ppb level). Under these conditions the time needed for sample preparation was about 15 min.

Fig. 5 shows the electropherograms corre-

sponding to tap water alone and spiked at 0.5 ppb.

4. Conclusions

CZE could be advantageously used in the monitoring of pentachlorophenol in water samples. Sample stacking techniques together with the use of extended light path capillaries allow detection limits to be obtained that are low enough to make this technique suitable even for drinking water. Direct injection could be useful for water samples having PCP concentrations higher than 2 ppm. Sample stacking without matrix removal can be used in many environmental monitoring studies. Sample stacking with matrix removal together with off-line SPE concentration using GCB cartridges is the method of choice for drinking waters. Quantification limits of 60 ppt can be attained by this procedure using reasonable small sample volumes (100 ml) which can be handled easily. Up to 1500 ml of sample can be processed, however, when necessary.

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