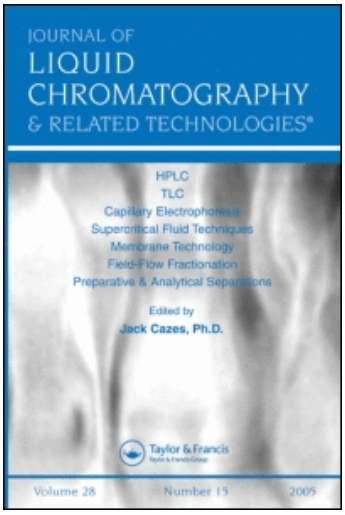

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Design of a Simple Detection Cell with Extended Optical Path Length for Capillary Electrophoresis: Application to Multiresidue Pesticide Analysis

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Abstract: Absorbance detection in capillary electrophoresis (CE), offers an excellent mass sensitivity, but poor concentration detection limits owing to very small injection volumes (normally 1 to 10 nL). This aspect can be a limiting factor in the applicability of CE/UV to detect species at trace levels, particularly pesticide residues. In the present work, the optical path length of an "on-column" detection cell was increased through a proper connection of the column (75 μm i.d.) to a capillary detection cell of 180 μm optical path length in order to improve detectability. It is shown that the cell with an extended optical path length results in a significant gain in terms of signal to noise ratio. The effect of the increase in the optical path length has been evaluated for six pesticides, namely, carbendazim, thiabendazole, imazalil, procymidone, triadimefon, and prochloraz. The resulting optical enhancement of the detection cell provided detection limits of ca. 0.3 μg/mL for the studied compounds, thus enabling the residue analysis by CE/UV.

Keywords: Pesticide residue analysis, Capillary electrophoresis (CE), Detection cell, CE/UV

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INTRODUCTION

Capillary electrophoresis (CE) in its various modes of operation has proven to be a resourceful alternative to the analysis of a great variety of solutes.[1] Initially introduced as a technique for the separation of biological macromolecules, CE has attracted much interest in other application areas, including pesticide-residue determination.[2] One of the greatest advantages is that no organic solvents are used in the preparation of the running buffer. Organic solvents are used as modifiers, but when this is done the levels only reach 5–30% of the total solvent. This is extremely cost effective since waste disposal is expensive as well as environmentally unsound. Some other advantages of CE include small sample volume, automation, as well as decreased cost of capillaries when compared to HPLC columns or GC capillary columns. CE also can operate in numerous modes, such as MECK and isotachophoresis, as well as free zone CE. This allows for numerous possibilities when trying to separate a variety of compounds.[3]

The commercial instrumentation currently provides detection options that basically include UV/visible absorbance and fluorescence. Despite the high sensitivity offered by fluorescence detection (minimum detectable concentration of ca. 10^{-8} M using conventional light sources and ca. 10^{-12} M with a laser source), the technique usually requires cumbersome chemical derivatization strategies that are impractical for routine analysis of a broad range of analytes. Furthermore, derivatization of these analytes at low concentrations results in poor yields and multiple impurities. The UV detector typically yields minimum detectable concentrations of ca. 10^{-6} M; because of their broad applicability. They are relatively inexpensive and can be focused down to the dimensions of the capillary, remaining the most popular detection mode.[4,5]

Detection in CE is mass sensitive because it measures the absolute on-column amount of analyte, but the concentration limits of detection are high because very small injection volumes (normally 1 to 10 nL) are used for analysis.[3,4] To enhance the detection capability of CE, several sample pre-concentration techniques or improved detection systems have been developed.[6–9] Among various sample pre-concentration techniques there is one major approach concentration through solid phase extraction (SPE).[10,11]

In CE, the loss in resolution between neighboring zones and/or zone overlap in the detection window can almost be neglected due to the micro optical path.[1,6] While quite universal and satisfactory for many applications, UV absorbance has a major problem in the detection of species at trace levels, particularly, pesticide residue.

Although the UV detector is reliable, easy to handle, and maintain, the relatively low sensitivity (10^{-5}–10^{-6} M) from the short optical path-length (5–100 mm) is an intrinsic drawback. This problem can be partially overcome by using the developed high-sensitivity detection cell (z-shape or bubble detection cell). The high-sensitivity detection cell has an optical

path-length of 1.2 mm and has been shown to increase the peak height of phenylurea herbicides.[12]

A bubble-shaped cell to extend the optical path length was made by forming an expanded region, a bubble, directly on the capillary column. This approach yielded a 3-fold increase in signal compared to the normal on-column detection.[13] The effect of the increase in optical cell path length has been evaluated through various capillary cell geometries and compared to commercially available detector cells.[14,15]

In this work, a simple procedure for increasing the minimum detectable concentration of UV-absorbance detectors is described. The optical path of the detection cell was increased through a proper connection of the analytical column (75 μm i.d.) with a capillary detection cell of 180 μm path length, demonstrating that an extended path length can result in a significant signal gain. The new detection cell was tested in the multiresidue analysis of pesticides Carbendazim, Imazalil, Prochloraz, Procymidone, Thiabendazole, and Triadimefon (Figure 1).

EXPERIMENTAL

Construction of the Detector Cell

In order to evaluate dispersion, band broadening, and effect of increasing path length, a "pseudo on column" detection cell was constructed as described

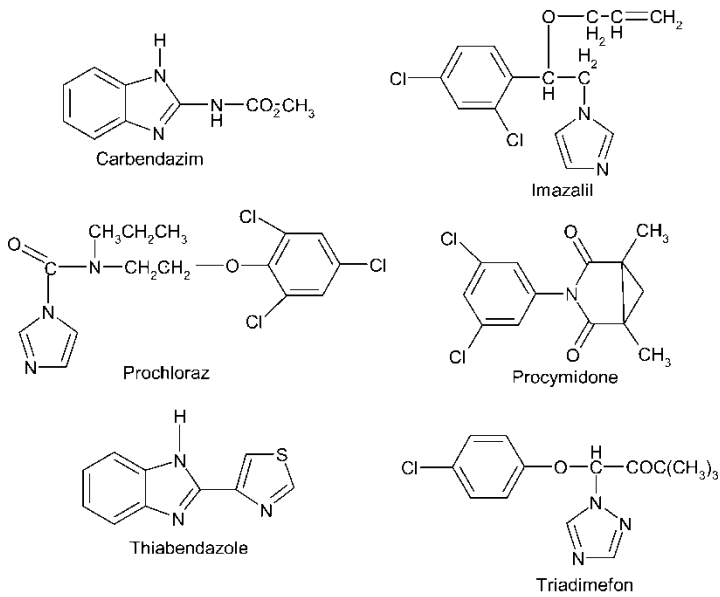

Figure 1. Names and structures of the test pesticides.

above. A schematic diagram of the proper detector cell design, constructed for this work is shown in Figure 2.

The detection system hardware was modified through a direct connection of a short piece of fused silica tube, measuring 334 mm in length and different internal diameters (100 and 180 μm i.d.) to the analytical column prepared as a "pseudo on column" detection cell. The "pseudo on column" detection cell was connected through a proper interface, using shrinkable Teflon tubing, to an analytical column of fused silica of 75 μm i.d × 1.00 m long. Conventional on-column detection was used to test 50 and 75 μm i.d analytical columns.

For the construction of the cell detector, the external coating (polyimide) of the fused silica was removed for a length of ca. 14 mm through heating, and solvent cleaned before being inserted into the template. The dead space at the joint is made as small as possible. This method allows the simple preparation of detection cells of various sizes by using fused silica tubes of different inner diameters. The volume of such a micro detection cell is about 0.15–1.2 nL.

A set of parameters could be characterized as the hardware of the detection system.[16] They are the parameters that should be controlled during the construction and installation of the detection cell:

- flow cell internal and external diameter (it is assumed to be part of a fused quartz column);
- incident beam width;
- convergence/divergence in the incident light beam (this is generally determined for a given detector, and difficult to influence by simple means);
- position and width of the photosensitive device;
- alignment of light beam, flow cell, and photocell.

Linearity

For quantitative work it is desirable that the output signal of the detector is linearly related to the solute concentration. Scott[16] has given the function:

$$y = A\ c^r \tag{1}$$

where y is the detector response, c is the sample concentration, A is a constant, and r is the response index. This formula is useful for determining the linearity of the detector and to provide numerical values that indicate the extent to which the function deviates from linearity. Thus, the slope of the equation is:

$$\log\ y = \log\ A + r\ \log\ c \tag{2}$$

gives the response index r. For a truly linear detector, $r = 1$ and the proximity of r to unity indicates the extent to which the detector deviates from true linearity. Scott[17] arbitrarily stated that linearity can be assumed only for values of r in the range $0.98 \leq r \leq 1.04$. However, it should be emphasized,

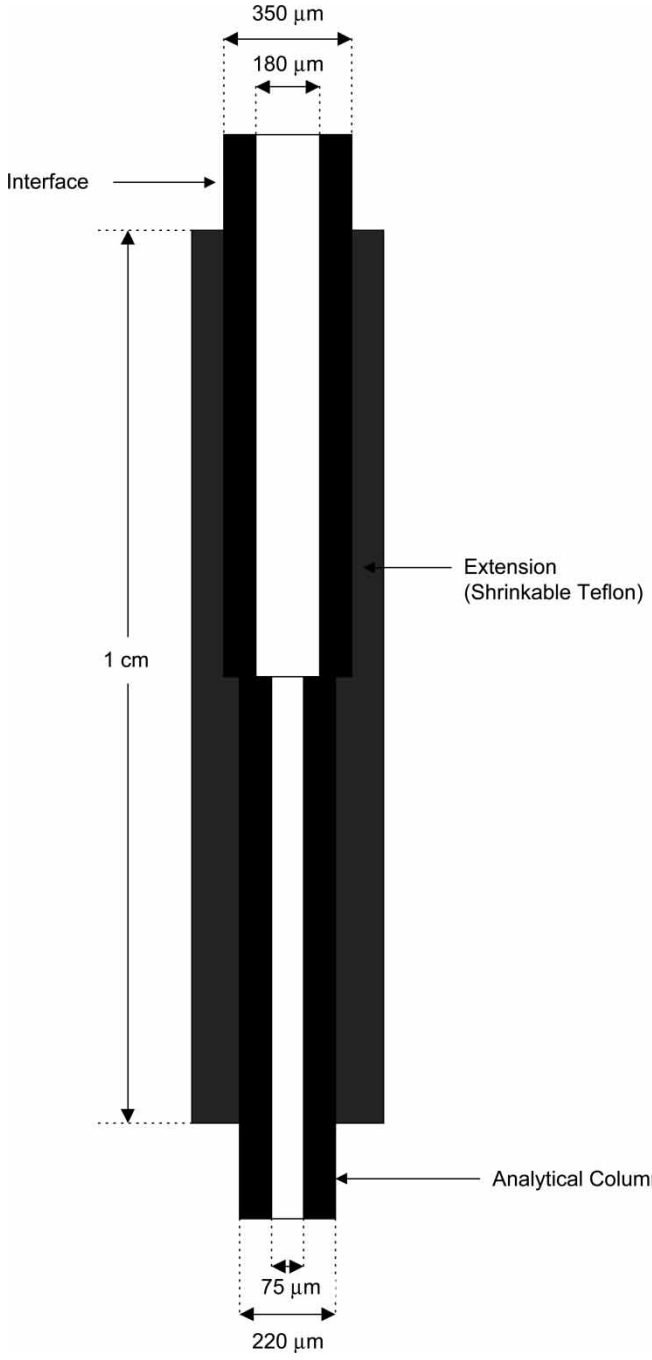

Figure 2. Schematic diagram of the connection from CE column to "pseudo on-column" detection cell.

that "non-linear" detectors can still be used, as long as r is known with sufficient accuracy (i.e., calibration curve).

In this work, linearity evaluation of "pseudo on-column"detection cell was carried out through parameters such as: linear work range, correlation coefficients, and response index. The linear parameters of work range and correlation coefficients were determined by plotting the peak area vs. concentration or peak mass of target analyte, and the response index was obtained by Equation (2) by plotting logarithmic format using a least square fit. The pesticide standards were dissolved in the running buffer starting with a concentration below the observable detection limit. Progressively higher concentrations were injected as triplicates onto the column, over the full sensitivity range of the detector.

CE System

The instrumentation consisted of a HP3D CE capillary electrophoresis system (Hewlett Packard, USA) equipped with a UV detector. The applied potential, as well as electrophoretic zones and the running buffer, were selected to give the best conditions of baseline separation and sensitivity for the multiresidue pesticide analysis. Sample introduction was done by hydrodynamic injection for 10 seconds. The columns used for the separation were made of fused silica tubes of 1.00 m long and 50 and 75 μm i.d. without any internal coating, being conditioned with a running buffer for 5 min before analysis. At the beginning of each day, the column is rinsed for 10 min with 0.1 M of NaOH, followed by 5 min with deionized water, and the running buffer for 5 min. At the end of each day, the capillary is rinsed for 10 min with 0.1M NaOH, followed by a 5-minute rinse with deionized water.

The optimum conditions chosen for this separation were obtained from tests with three different buffers: phosphate, tetraborate, and carbonate, and sodium dodecyl sulphate (SDS) and sodium cholate as surfactants. The separation was performed with a positive power supply of 15 kV at 25°C, using 50 mM sodium tetraborate containing 70 mM sodium cholate and detection wavelength in 210 nm. The typical current during the analysis was 48 μA.

RESULTS AND DISCUSSION

Evaluation of the Detection System

In the past decade, numerous studies were devoted to the miniaturization of conventional "Z type" detection cells that demonstrated a good approach to increasing the sensitivity in HPLC. Direct application of this kind of cell and design, however, in the case of CE is limited, mainly because of the low injection volume (nL), as well as by the relatively high background

noise caused by the low light transmission through the bent section of the capillary.

In this work, the optical path length of an "on-column" detection cell was increased through a proper connection of the column (75 μm i.d.) to the detection cell of higher optical path length, in order to improve detectability. This detection system was named "pseudo on-column" to differ from the conventional on-column detection.

The evaluation of an alternative "pseudo on-column" detection was carried out in relation to the linearity of the system by plotting the peak area vs. analyte concentration. The linear work range, correlation coefficients (based on individual analytical curve), as well as response index (based on Scott's Equation (2) found for compounds studied in several detection systems are listed in Table 1.

Response indexes found through Scott's Equation (2) were determined by plotting the logarithmic format using a least square fit. Values of response indexes obtained (Table 1) ranged from 0.89 to 0.96, depending on the solutes studied. Experimental tests, even with response index values, were out of the range proposed by Scott; good correlation coefficient results were obtained from the calibration curves (0.9982 to 0.9990). These results indicated that calibration curves can be used with good accuracy. Since

Table 1. Linearity data for the studied pesticides in several detection cells

		Detection cell i.d. (μm)			
Pesticides	Linearity parameters	50	75	100	180
Carbendazim	Response index	0.89	0.90	0.92	0.90
	Work range (μg/mL)	4–40	3–30	2–20	1–10
	Correlation coefficient	0.9984	0.9982	0.9989	0.9990
Thiabendazole	Response index	0.88	0.91	0.91	0.94
	Work Range (μg/mL)	2–20	1.5–20	1.0–10	0.60–6
	Correlation coefficient	0.9986	0.9990	0.9989	0.9995
Procymidone	Response index	0.88	0.92	0.93	0.92
	Work range (μg/mL)	2–20	1.2–15	1.0–10	0.3–5
	Correlation coefficient	0.9993	0.9990	0.9996	0.9995
Imazalil	Response index	0.90	0.91	0.90	0.90
	Work range (μg/mL)	1.5–2	1–10	0.6–6	0.30–5
	Correlation coefficient	0.9985	0.9991	0.9994	0.9997
Triadimefon	Response index	0.89	0.92	0.94	0.92
	Work range (μg/mL)	2–20	1.2–15	1–10	0.5–5
	Correlation coefficient	0.9987	0.9991	0.9995	0.9996
Prochloraz	Response index	0.87	0.90	0.91	0.94
	Work range (μg/mL)	1.5–15	1–10	0.6–6	0.3–5
	Correlation coefficient	0.9988	0.9990	0.9994	0.9995

this work aims at evaluating the CE capability in the multiresidue pesticide analysis, the work mass range assessed was in terms of low concentration. The results shown in Table 1, concern individual calibration curves for the analytes, and point out that the 180 μm "pseudo-on-column" detection cell is most suitable for this purpose.

Sensitivity

The sensitivity evaluation was performed in terms of the minimum detectable amount, which is normally considered as the amount that produces a peak area or absorbance three times the signal/noise relation considering the maximum sensitivity range.[18,19] In addition, the reproducibility was evaluated at the minimum concentration detection, using the relative standard deviation (RSD) and standard deviation (SD) methods.

Table 2 presents the minimum detectable amount values for six pesticides, as well as the RSD and SD for each detection cell studied. Since the injection mode utilized during the entire study was hydrodynamic for 10 seconds, it represents about 10 nL of the injection volume. The results obtained for SD and RSD are nearly satisfactory for capillary electrophoresis, ranging from 3 to 5%. The influence of increasing the i.d. of the detection cell over the minimum detectable amount (MDA) can be observed in Table 2, showing lower amounts for the higher optical path length represented by the 180 μm i.d. pseudo on-column detection cell.

The MDA values found for the multiresidue pesticide analysis using an 180 μm i.d. "pseudo on-column" detection cell showed a good approach for this sort of analysis, presenting good linearity and low detection levels (0.3 μg/mL – 1.0 μg/mL).

Figure 3 shows representative electropherograms obtained for multiresidue pesticide analyses identified, with (b) illustrating the enormous gain

Table 2. Experimentally observed detection limits (μg/mL) for the studied pesticides in several detection systems

	Detection limits (μg/mL) (RSD)[a]			
Pesticides	50	75	100	180
Carbendazim	4.35 (5.3)	2.90 (3.8)	2.00 (4.3)	1.00 (3.7)
Thiabendazole	2.35 (4.8)	1.50 (3.7)	1.10 (3.9)	0.60 (3.6)
Procymidone	1.90 (4.4)	1.20 (4.5)	0.90 (3.8)	0.50 (3.9)
Imazalil	1.40 (4.5)	0.90 (4.1)	0.60 (3.5)	0.30 (3.2)
Triadimefon	1.90 (4.4)	1.20 (3.3)	0.90 (3.7)	0.50 (3.7)
Prochloraz	1.40 (5.1)	0.90 (4.4)	0.60 (4.2)	0.30 (4.2)

[a]The Relative Standard Deviation (RSD %) was based on triplicate experiments.

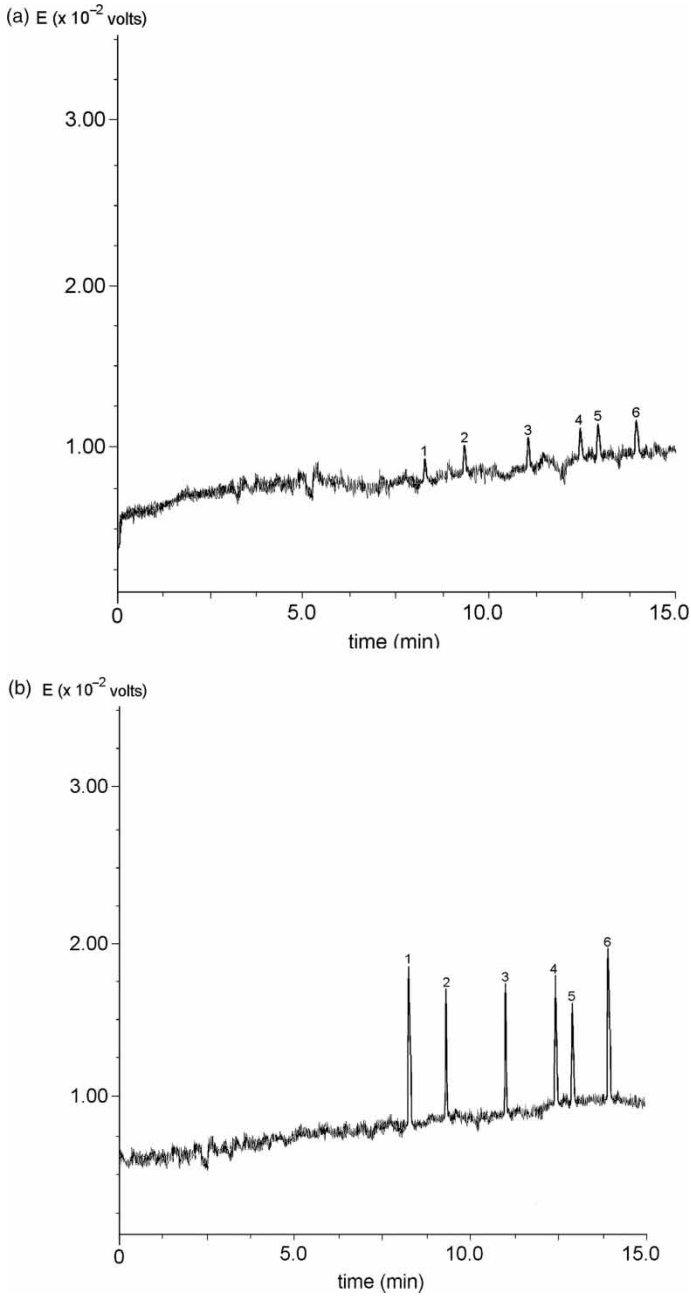

Figure 3. Electropherograms of multiresidue pesticide at 5 μg/mL in sodium tetraborate 50 mM containing 70 mM sodium cholate using "pseudo on-column" detection cell with (a) 75 μm i.d. and (b) 180 μm. Peak identification: (1) carbendazim; (2) thiabendazole; (3) procymidone; (4) imazalil; (5) triadimefon; (6) prochloraz.

based on the peak area for 180 μm optical path length cell compared with (a) that represents a 75 μm optical path length cell. The results obtained showed that the system tested using "pseudo on-column" detection cell greatly extends the scope of CE as an analytical technique, improving the detectability required for trace analyses.

CONCLUSIONS

Several important conclusions can be drawn from our results that are vital to the increased optical path length of the UV detection cell in capillary electrophoresis:

- It is possible to enhance the detection sensitivity by mean of a "pseudo on-column" detection cell connecting it directly to the column.
- The increase of the inner diameter for the tested "pseudo on-column" improved mass sensitivity for UV detection in the CE system.
- The best results of linearity, taking into account parameters such as response index, linear work range, and correlation coefficients were obtained using the 180 μm i.d "pseudo on-column" detection cell.
- The detection limits obtained using a 180 μm i.d. cell were 0.3 μg/mL to 1.0 μg/mL.
- The increased mass sensitivity in CE separations through an improvement of the optical path length, as described here, makes possible its use as a powerful technique in the multiresidue pesticide analyses field.

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