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On-column trace enrichment by sequential frontal and elution electrochromatography
II. Enhancement of sensitivity by segmented capillaries with z-cell configuration—application to the detection of dilute samples of moderately polar and nonpolar pesticides☆

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Abstract

An on-column trace enrichment method for capillary electrochromatography of dilute samples is described. It involves the sequential use of frontal and elution electrochromatography on a segmented capillary column comprising of two contiguous segments each packed with a different sorbent. While the entering segment is for preconcentration by frontal electrochromatography the second segment is much longer and is meant for separation of the enriched analytes in the subsequent elution electrochromatography step. The preconcentration segment is usually packed with a sorbent that affords the highest affinity towards the solutes of interest while the separation segment is packed with a stationary phase that exhibits the highest selectivity and separation efficiency for the analytes. The detection is performed in the UV using a z-cell configuration for achieving an increased path length for detection. The effectiveness of this on-column trace enrichment is demonstrated on dilute samples of moderately polar solutes (e.g., carbamate insecticides) and nonpolar solutes (e.g., pyrethroid insecticides). Under optimal frontal and elution electrochromatography conditions, 817- and 1100-fold sensitivity increase are achieved for permethrin (a pyrethroid insecticide) and methiocarb (a carbamate insecticide), respectively, with a UV detector. The method is demonstrated with real water samples (e.g., tap and lake water samples) spiked with carbamate and pyrethroid insecticides. The limits of detection for the pesticides achieved in tap and lake waters reached 10^{-8} to 10^{-9} *M*.

Keywords: Electrochromatography; Sensitivity; Sample handling; Pesticides; Carbamates; Pyrethroids

1. Introduction

As with other microcolumn separation techniques, capillary electrochromatography (CEC) suffers from its rather poor concentration sensitivity due to the small sample volume and the short path length for on-column photometric detection. This fact is the major drive for introducing new ways for enhancing

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CEC concentration sensitivity and enabling the technique to analyze dilute samples. The alleviation of this shortcoming is essential for the exploitation of the high resolving power of CEC in the analysis of dilute samples of biological and environmental provenance in order to meet current challenges such as those imposed by the era of proteomic and safe drinking water whereby low abundance proteins must be isolated and characterized and trace amounts of toxic pollutants must be determined, respectively. Although extensive research efforts have been dedicated to improving detection sensitivity in capillary electrophoresis by developing various approaches for on-line preconcentration (for recent reviews, see Refs. [1–4]), only a few reports have appeared so far concerning on-column trace enrichment for enhancing detection sensitivity in CEC [5–9]. The on-line preconcentration approaches used in capillary electrophoresis (CE) are several including field amplified sample stacking, large volume sample stacking, pH-mediated focusing, isotachophoretic stacking, chromatographic extraction and sweeping [1–4]. The approaches used in CEC are based on the capability of the chromatographic column packed with microparticles to play the simultaneous role of preconcentrator and separator [5–9].

This report is a continuation to our earlier contribution to on-column trace enrichment of dilute samples in CEC [6,7,9]. In our initial studies [6,7], a water plug of a few millimeters long (3 to 5 mm) was injected first followed by prolonged injection of the dilute sample (ca. 12 mm sample plug) dissolved in a solvent of the same composition as the mobile phase used in the separation step. The water plug (the more retentive mobile phase) brought about an enhanced accumulation of the dilute samples into a narrow band at the inlet of the CEC column. This approach allowed a maximum sensitivity enhancement of 100-fold. Very recently, our approach to on-column trace enrichment involved sequential frontal and elution electrochromatography whereby relatively long sample plug (21 cm) dissolved in a solvent of weaker eluting strength than the mobile phase is first introduced followed by elution with a mobile phase of stronger eluent strength thus effecting a step-gradient elution and zone narrowing in the separation step [9]. This approach allowed a 500-fold sensitivity enhancement. To further boost the concentration sensitivity of CEC, we are describing here the sequential frontal and elution electrochromatography with a segmented capillary and a z-cell configuration for detection. The present approach allowed a sensitivity enhancement of 817- and 1100-fold for permethrin (a pyrethroid insecticide) and methiocarb (a carbamate insecticide), respectively. In the segmented capillary configuration, the capillary column consists of two contiguous segments each packed with a different stationary phase: the inlet segment is a short segment for preconcentration and the second segment is a longer segment and functions as the separation segment. While the preconcentration segment should provide the highest affinity towards the enriched analytes, the separation segment should exhibit the highest selectivity and separation efficiency.

2. Basic principles

The basic principles of the on-column trace enrichment method used in this study were previously described in detail [9]. Briefly, they comprised two distinct steps: a frontal electrochromatography (FEC) step followed by an elution electrochromatography (EEC) step. In the FEC step, the dilute sample is applied to the column in a relatively large volume under conditions of strong solute binding to the stationary phase. Under these circumstances, solute molecules will accumulate at the column entrance in a relatively narrow band (i.e., zone compression or narrowing), see Fig. 1. After this stationary phase mediated focusing step, the solutes molecules are swept with a mobile phase whose organic modifier content is relatively high to bring about the rapid elution and separation (i.e., elution electrochromatography by a step gradient) of the enriched solute band, see Fig. 1. This step gradient brings about a focusing effect on the enriched solute band.

The effectiveness of the on-column trace enrichment is largely influenced by the affinity of the individual solutes to the stationary phase during the FEC step. The stronger the retention of the solute the narrower the concentrated band at the top of the column. It can be demonstrated that under a given mobile phase linear velocity (i.e., electroosmotic flow velocity), the length of the enriched solute band

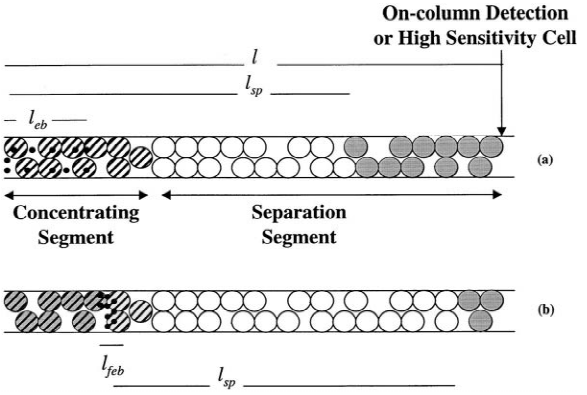

Fig. 1. Schematic illustration of trace enrichment by frontal electrochromatography in (a) followed by zone focusing during step gradient by elution electrochromatography. This illustration also shows the segmented capillary design consisting of two contiguous segments packed with different stationary phases: a concentrating segment preceding a separation segment. l, Length of column to detection point; l_{eb}, length of enriched band; l_{sp}, length of sample plug; l_{feb}, length of focused enriched band.

or compressed solute zone l_{eb} is given by the product of the length of the sample plug l_{sp} and the retardation factor $1/(1+k')$ as follows [9]:

$$l_{eb} = l_{sp} \cdot \left(\frac{1}{1+k'}\right) \quad (1)$$

where k' is the retention factor of the solute being enriched in the injection solvent. For a given sample plug length, Eq. (1) shows that the effectiveness of zone compression (i.e., very small l_{eb}) during enrichment increases with increasing k'. It is obvious that the thinner the enriched band (i.e., the smaller l_{eb}) the stronger the signal intensity and the more effective the on-column trace enrichment. To ensure an optimal on-column trace enrichment, the high retention factor during injection (i.e., during FEC step) must be combined with negligible retention during desorption (i.e., during EEC step) in order to minimize band broadening during elution. This is usually accomplished by combining a strong eluent (i.e., an organic-rich mobile phase) with a relatively strong EOF which can be achieved by a relatively high running voltage, a column with high surface charge density, and a mobile phase of low viscosity.

As shown in Fig. 1, we have devised a segmented capillary column configuration with a z-cell in order to accomplish the highest performance in terms of trace enrichment and detection sensitivity. In this configuration, while the concentrating segment provides strong solute binding during FEC step (i.e., the thinnest l_{eb}), the separation segment provides the selectivity and plate counts during EEC step. The length of the concentrating segment need not to exceed the length of the enriched solute band l_{eb} (i.e., 3 to 5 mm). But for convenience and ease of packing the capillary column, we have set this length in our work to 2.5 cm which is about 10% of the effective length of capillaries used in our studies. For details of packing segmented capillaries, see the Experimental section and also Ref. [10]. Previously, we have introduced the concept of segmented capillaries for the control of electroosmotic flow (EOF) in CE [11,12] and CEC [10] separations. In these works, we have shown that the overall EOF in segmented capillaries is a linear function of the fractional length of a given segment as follows:

$$\mu_{eo,av} = (\mu_{eo,1} - \mu_{eo,2}) \cdot \frac{l_1}{l_t} + \mu_{eo,2} \quad (2)$$

where $\mu_{eo,1}$ and $\mu_{eo,2}$ are the electroosmotic mobilities in segment 1 (e.g., concentrating segment) and segment 2 (e.g., separation segment), respectively, when operated individually. l_t, l_1 and l_2 are the total column length, the length of segment 1 and segment 2, respectively. The total column length is $l_t = l_1 + l_2$. Eq. (2) shows that knowing the individual EOF in each segment, the overall EOF in segmented capillaries can be readily predicted.

During the EEC step, a step-gradient elution occurs where the enriched sample band is further compressed or stacked yielding a band of length l_{feb}, see Fig. 1. This stacking comes about from the fact that the solute molecules contacting the eluting mobile phase travel much faster than the solute molecules on the opposite side contacting the injection solvent which has a weaker eluent strength. The net result is an additional zone focusing until the eluting mobile phase reaches the slow migrating side of the injected enriched band. This zone focusing (i.e., zone narrowing) increases with (i) increasing the difference in the eluent strength between the injection solvent (weak eluent, high k') and the mobile phase (strong eluent, low k') and (ii) decreasing the length of the sample plug (l_{sp}).

3. Experimental

3.1. Instrumentation

The instrument used was a HP^{3D} CE system from Hewlett-Packard (Waldbronn, Germany) equipped with a photodiode array detector. Electrochromatograms were recorded with a computer running HP^{3D} CE ChemStation. A pressure of 10 bar was applied to both ends of the capillary during the experimental runs. The temperature was held constant at 25 °C. All samples were injected electrokinetically at various times and applied voltages which are stated in figure captions.

3.2. Reagents and materials

Buffer solutions were prepared using either Tris from Fisher Scientific (Fair Lawn, NJ, USA), sodium phosphate monobasic from Mallinckrodt (Paris, KY, USA) or ammonium acetate from EM Science (Gibbstown, NJ, USA). The organic modifier used in the mobile phase was HPLC-grade acetonitrile (ACN) or tetrahydrofuran (THF) purchased from Fisher Scientific. All solutions were passed through a 0.2-μm Titan syringe filter obtained from Scientific Resources (Eatontown, NJ, USA). The insecticides (see Fig. 2) were purchased from ChemService (West Chester, PA, USA). Nucleosil silica was purchased from Macherey–Nagel (Düren, Germany). Fused-silica capillaries of 100 μm I.D.×360 μm O.D. were from Polymicro Technology (Phoenix, AZ, USA).

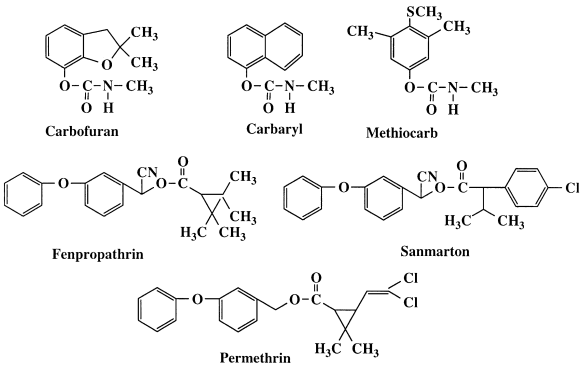

Fig. 2. Structures of the *N*-methylcarbamate insecticides (carbofuran, carbaryl and methiocarb) and pyrethroid insecticides (fenpropathrin, sanmarton and permethrin) used in this study.

3.3. Stationary phases and column packing

Four types of nonpolar silica-based stationary phases were examined in this study. The first stationary phase was based on a 5 μm Nucleosil 120-5 silica with an average pore diameter of 120 Å and a specific surface area of 200 m^2/g. This silica was converted in the laboratory to an octadecyl-silica (ODS) according to our previously described procedures [9,13]. The second stationary phase investigated was 5 μm Nucleosil C_{18} silica from Macherey–Nagel having an average pore diameter of 100 Å and a specific surface area of 250 m^2/g. This stationary phase is endcapped with a carbon coverage of 15% (w/w) as stated by the manufacturer. The third sorbent was a 5 μm ODS with an average pore diameter of 120 Å and came from J.T. Baker (Phillipsburg, NJ, USA). The fourth silica was a 5 μm Zorbax C_8 from DuPont (Wilmington, DE, USA).

Untreated fused-silica capillaries were packed using the slurry packing technique [9,13]. All capillaries used in this study were 25 cm packed/33.5 cm in total length. See Fig. 1 for the configuration of segmented capillaries. When packing capillaries with two segments (each segment with a different stationary phase), the concentrating segment was first packed to an approximate length of 8 cm. A pressure of 200 bar was applied and the silica length was measured to the nearest millimeter. Following, the separation segment was packed at the same pressure as the concentrating segment. Thereafter, the concentrating segment was cut to the desired length followed by sintering the inlet frit. Throughout the length of the concentrating segment was 2.5 cm. The outlet frit was sintered to form an overall effective length (i.e., to the detection point) of 25 cm. When using the high sensitivity cell, the capillary was cut at 5 mm after the outlet frit. When using on-column detection, the outlet end of the capillary was cut at 8.5 cm after the outlet frit. The remaining silica in this 8.5 cm segment was flushed out using an HPLC pump, and the detection window was formed 5 mm from the outlet frit.

To acquire a greater sensitivity a HP^{3D} CE high-sensitivity detection cell was employed. The z-shape of the cell allows for a 1200 μm path length as compared to the 100 μm path length of a regular capillary column. The increased path length can lead to a sevenfold increase in sensitivity when compared to the 100 μm capillary [14].

3.4. Procedures

Stock solutions of three carbamate (i.e., carbofuran, carbaryl, and methiocarb) and three pyrethroid insecticides (i.e., fenpropathrin, sanmarton and permethrin) were prepared by dissolving 10 mg of each insecticide in individual 100 ml volumetric flasks filled with HPLC-grade acetonitrile. This gave a concentration of $2\cdot10^{-4}$ to $5\cdot10^{-4}$ *M* depending on the insecticides. A first dilution was performed by taking 1.0 ml of the stock solution and diluting it to 100 ml with HPLC-grade acetonitrile in a volumetric flask to yield an approximate concentration of $4\cdot10^{-6}$ *M*. The solvent used in the last dilution step consisted of pure buffer or various mixtures of acetonitrile–buffer (10:90 to 80:20, v/v). Since the insecticides were dissolved in pure acetonitrile, the stock solution pipetted was kept small (e.g., 10 μl or less) as not to affect the overall concentration of acetonitrile in the final dilution.

4. Results and discussion

4.1. Capillary configuration—selection of stationary phases best suited for preconcentration and separation

Four different non-polar stationary phases were first examined for their plate counts with alkylbenzenes as the test solutes as well as for their EOF using a running voltage of 20 kV and a mobile phase of ACN–water (4:1, v/v) whose aqueous component (10 m*M* ammonium acetate) had a pH of 6.0. The laboratory-made ODS Nucleosil stationary phase provided the highest separation efficiency (123 400 plates/m) as well as a relatively fast EOF (1.21 mm/s). This ODS stationary phase was intentionally prepared at a relatively low surface coverage in octadecyl ligands [9] to ensure a relatively strong EOF. Under the same running conditions and for the same test solutes, the capillary packed with Zorbax C_8 provided a slightly higher EOF velocity of 1.35 mm/s but a significantly lower separation efficiency of 67 000 plates/m. The capillaries packed with the ODS from J.T. Baker or the endcapped ODS Nucleosil from Machery–Nagel provided the slowest EOF velocities of 1.02 and 0.91 mm/s, respectively, with an average plate counts of 78 000 and 67 000 plates/m, respectively. Besides the laboratory-made ODS, which was specially designed for use in CEC, the other three non-polar silica-based stationary phases were manufactured for use in HPLC. From the above experiments, it is obvious that the stationary phase best suited for separation is the laboratory-made ODS Nucleosil because it exhibited the highest separation efficiency with a relatively strong EOF.

In order to select the stationary phase best suited for preconcentration, the various stationary phases were evaluated with the pesticides under investigation. Three carbamate insecticides (namely carbofuran, carbaryl and methiocarb) and three pyrethroid insecticides (namely fenpropathrin, sanmarton and permethrin) were analyzed at various acetonitrile contents of the mobile phase. The results are shown in Figs. 3a and 4a in terms of log k' vs. % ACN in the eluent. The plots shown in Figs. 3a and 4a are for carbofuran and permethrin, respectively, as representative solutes of *N*-methylcarbamate and pyrethroid insecticides, respectively. In all cases, linear plots were obtained. Since the carbamate insecticides are relatively polar solutes, it is not surprising to see that the laboratory-made ODS Nucleosil (of low surface coverage in octadecyl ligands) and the Zorbax C_8 yielded the highest retention toward the carbamate insecticides. In both cases, the unreacted surface silanols are most likely contributing to enhanced solute retention via silanophilic interactions. Using these data, the lengths of the compressed solute bands l_{eb} during the FCE step were calculated from Eq. (1) at the various % ACN in the injection solvent. Plots of l_{eb} versus % ACN for the various stationary phases are shown in Fig. 3b for the representative solute methiocarb. As can be seen in this figure either Zorbax C_8 or the laboratory-made ODS Nucleosil are ideal for use in the preconcentration segment in segmented capillaries depending on the % ACN used in the injection

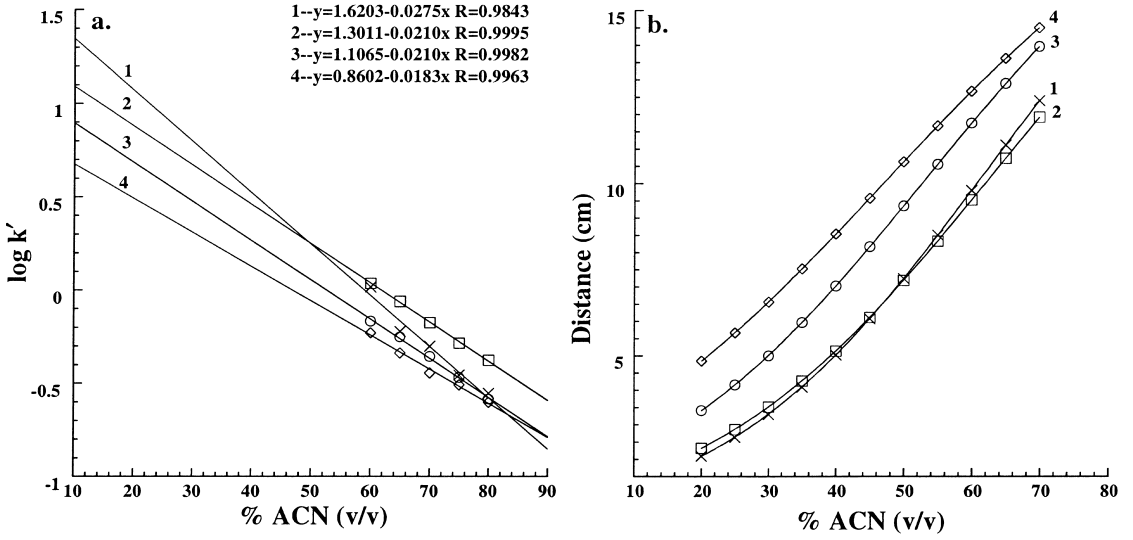

Fig. 3. (a) Plots of logarithmic retention factor (log k') for carbofuran vs. % (v/v) acetonitrile in the mobile phase and (b) plots of the compressed band of carbofuran l_{eb} vs. % (v/v) acetonitrile in the injected sample. Conditions: capillary column, 33.5 cm (effective length 25 cm)×100 μm; mobile phase hydro–organic solution made up of various concentrations of CH_3COONH_4 to acquire an overall ammonium acetate concentration of 2 m*M* (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; column temperature 25 °C. (b) Data points calculated from Eq. (1) using l_{sp} = 21 cm and k' values at different % ACN. 1, Zorbax C_8; 2, laboratory-made ODS Nucleosil; 3, J.T. Baker C_{18}; 4, Nucleosil 100-5 C_{18}.

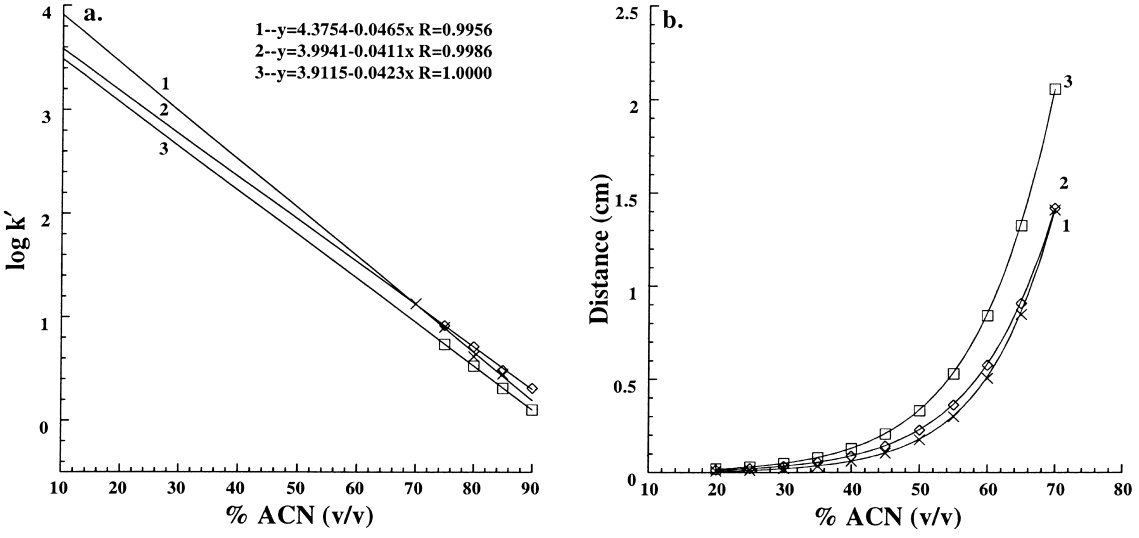

Fig. 4. (a) Plots of logarithmic retention factor (log k') for permethrin vs. % (v/v) acetonitrile in the mobile phase and (b) plots of the compressed band of permethrin l_{eb} vs. % (v/v) acetonitrile in the injected sample. Conditions: capillary column, 33.5 cm (effective length 25 cm)×100 μm; mobile phase hydro–organic solution made up of various concentrations of CH_3COONH_4 to acquire an overall ammonium acetate concentration of 2 m*M* (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; column temperature 25 °C. (b) Data points calculated from Eq. (1) using l_{sp} = 21 cm and k' values at different % ACN. 1, Zorbax C_8; 2, Nucleosil 100-5 C_{18}; 3, laboratory-made ODS Nucleosil.

solvent. At relatively low % ACN (below 40%) Zorbax C_8 provides thinner l_{eb} while the laboratory-made ODS Nucleosil is the opposite and yields thinner l_{eb} at high % ACN.

On the other hand, since the pyrethroids are relatively more hydrophobic than the carbamates, the ODS with the highest surface coverage (i.e., the endcapped ODS Nucleosil from Machery–Nagel) provided higher retention than the laboratory-made ODS Nucleosil (see Fig. 4a) and in turn the thinnest compressed enriched band l_{eb} at all % ACN in the sample solvent (see Fig. 4b). Unexpectedly, the Zorbax C_8 exhibited the same or slightly higher retention toward the pyrethroids than the ODS with the high % C (Fig. 4a). This can be explained by the fact that the relatively short C_8 chains of the Zorbax C_8 do not ''stack'' as extensively as the longer octadecyl ligands on the ODS column (see Fig. 5 for illustration of the stack configuration). In other words, the effective nonpolar phase ratio available for solute interaction is about the same or higher on the Zorbax C_8 column than on the ODS column from Machery–Nagel with high carbon load. Since the ODS with high surface coverage yielded the lowest EOF, Zorbax C_8 was elected as the stationary phase for the preconcentration segment.

Previously, we have investigated the CEC behavior of carbamate insecticides as a function of the organic content of the mobile phase [9], and found that an eluent at 80 to 90% (v/v) ACN was very suitable for the rapid separation of the carbamate insecticides. Therefore, and for completeness, it was necessary to evaluate in this study the retention of pyrethroid insecticides in reversed-phase liquid chromatography (RPLC) with the laboratory-made ODS Nucleosil so that the amount of organic modifier in mobile phase needed for separation could be determined. Fig. 6a illustrates the separation of the three pyrethroids using a mobile phase of 14 m*M* ammonium acetate–THF–acetonitrile (15:5:80, v/v). As shown in Fig. 6b, linear plots of log k' versus percent acetonitrile in the mobile phase were obtained demonstrating that the stationary phase exhibited RPLC behavior toward the three pyrethroids studied. It was found that by adding 5% (v/v) THF to the mobile phase as compared to using acetonitrile alone decreased the k' for fenpropathrin from 1.5 to 0.9 and for permethrin from 2.3 to 1.6. This indicates that THF has a strong eluting power towards the pyrethroids. This strong eluting power is necessary in on-line trace enrichment for the sweeping, which occurs in EEC step after the injection or stacking during the FEC step so that the enriched band is reduced in size (i.e., zone compression) before the final separation step.

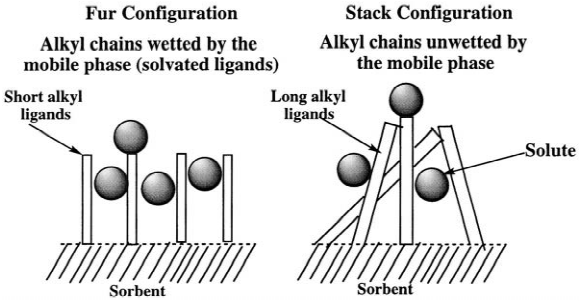

Fig. 5. ''Stack'' configuration versus ''fur'' configuration for alkylated stationary phases.

4.2. On-line trace enrichment

In a very recent contribution from our laboratory [9], the effects of the sample injection voltage during trace enrichment by FEC, the length of the sample plug and the composition of the mobile phase used in the elution step were investigated in order to find the conditions under which optimum trace enrichment can be performed [9]. We have shown that increasing the applied voltage during sample enrichment by FEC led to increasing signal intensity of the enriched band upon elution. This was attributed to increasing the magnitude of perfusive EOF (i.e., the flow within the pores) which lead to increased mass transport within the pores and consequently higher capacity and better focusing of the sample. An applied voltage of 20 kV for sample introduction was a good compromise. Also, the study of the effect of sample plug length revealed that the higher the solute retention in the FEC step, the longer the permissible sample plug length and consequently the higher the analyte signal in the subsequent elution electrochromatography step. As stated in Section 2, for achieving the most effective on-column trace enrichment, the FEC step should be combined with the fastest EEC step. This means that the larger the difference

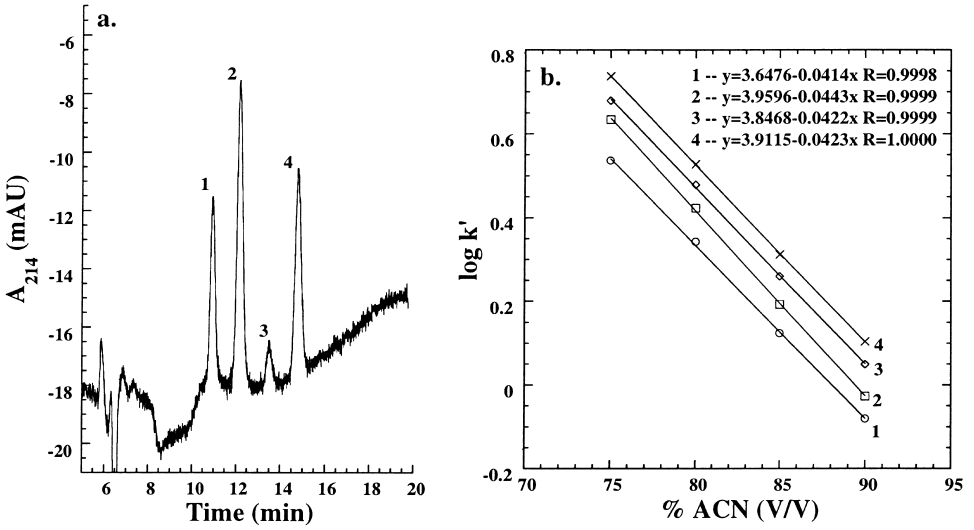

Fig. 6. (a) Electrochromatogram of pyrethroids used in this study and (b) plots of logarithmic retention factor (log k') of the three pyrethroids vs. % (v/v) acetonitrile in the mobile phase. Conditions: capillary column, 33.5 cm (effective length 25 cm)×100 μm; mobile phase: (a), hydro–organic solution made up of 14 m*M* CH_3COONH_4 (pH 6.0)–acetonitrile–THF (15:80:5, v/v). (b) Various concentrations of CH_3COONH_4 to acquire an overall ammonium acetate concentration of 2 m*M* (pH 6.0) mixed with acetonitrile at different proportions; voltage, 20 kV; detection wavelength, 214 nm; column temperature, 25 °C. Solutes: 1, fenpropathrin; 2, sanmarton; 3, *trans*-permethrin; 4, *cis*-permethrin.

between the eluent strength of the mobile phase and the injection solvent the better the effectiveness of the zone compression during the step-gradient elution.

4.2.1. Effect of acetonitrile content in the sample plug

Fig. 7a and b shows the effect of % ACN in the sample plug on the effectiveness of the on-line preconcentration by means of FEC on a single sorbent capillary and a segmented capillary, respectively, using permethrin as the model solute. The single sorbent capillary consisted of the laboratory-made ODS Nucleosil while the segmented capillary column was made of a Zorbax C_8 segment (2.5 cm) and laboratory-made ODS Nucleosil segment (22.5 cm). The results shown in Fig. 7a and b are expressed in terms of peak height in mAU versus % ACN in the sample plug. The sample plug was introduced electrokinetically at 20 kV from a sample solution at $1.3 \cdot 10^{-7}$ *M* in permethrin for an injection time of 3.5 min. The content of the sample in ACN was varied between 0 and 80% (v/v) for the segmented column (Zorbax $C_8 \rightarrow$laboratory-made ODS Nucleosil) while for the single-phase capillary column (packed with laboratory-made ODS Nucleosil) it was varied between 30 and 60% (v/v). On the average, the length of the sample plug (l_{sp}) was around 21 cm, which is about 84% of the effective length of the column. In all cases, the elution step was carried out with a mobile phase composed of acetonitrile–10 m*M* ammonium acetate, pH 6.0 (80:20, v/v), using 20 kV as the elution voltage. As can be seen in Fig. 7b, the peak height increases slowly first in the 0 to 20% ACN range and then rapidly in the 20 to 40% ACN range. Increasing the ACN content improves the surface contact with the hydro–organic solution being enriched by opening the hydrophobic chains (i.e., the octadecyl ligands), thus increasing the effective hydrophobic surface area, see Fig. 5. This is in agreement with the findings that in solid-phase extraction (SPE) with ODS cartridges, the SPE materials are usually pretreated or pre-wetted with an activating solvent such as ACN, MeOH or acetone to improve surface contact [15]. In the 0 to 50% ACN range, the solute accumulates at the entrance of the column occupying a distance l_{eb} of 0.0009, 0.003, 0.008, 0.02, 0.06 and 0.2 cm at 0, 10, 20, 30, 40 and 50% ACN in the sample plug, respectively. These values were esti-

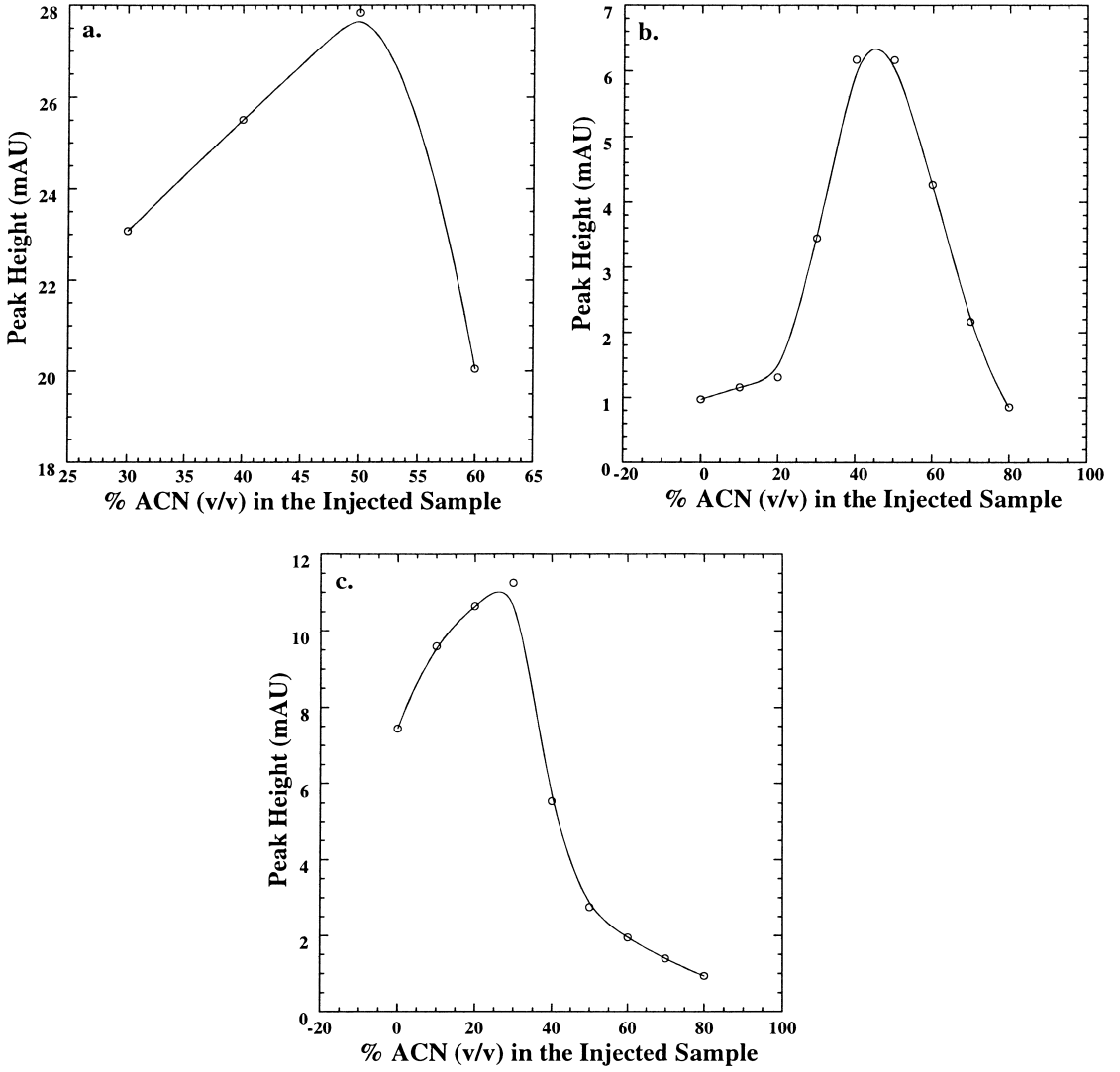

Fig. 7. (a) Plot of peak height of permethrin vs. % (v/v) acetonitrile in the injection sample for single sorbent capillary packed with laboratory-made ODS Nucleosil silica. (b) Plot of peak height of permethrin vs. % (v/v) acetonitrile in the injected sample for a segmented capillary column consisting of 2.5 cm Zorbax C_8 segment followed by 22.5 cm of laboratory-made ODS Nucleosil silica segment. (c) Plot of peak height of carbaryl vs. % (v/v) acetonitrile in the injected sample for a segmented capillary column consisting of 2.5 cm Zorbax C_8 followed by 22.5 cm of laboratory-made ODS Nucleosil silica segment. Conditions of frontal electrochromatography: organic in the injected sample, 30, 40, 50 and 60% ACN for (a) and 0, 10, 20, 30, 40, 50, 60, 70 and 80% (v/v) ACN for (b) and (c); sample plug was introduced electrokinetically at 20 kV from sample solution at $1.28 \cdot 10^{-7}$ *M* in permethrin in (a) and (b) or $1.28 \cdot 10^{-6}$ *M* in carbaryl in (c) for an injection time of 3.5 min. Conditions of elution electrochromatography: mobile phase, 10 m*M* CH_3COONH_4 (pH 6.0)–acetonitrile (20:80, v/v); voltage, 20 kV; detection wavelength, 214 nm for permethrin and 281 nm for carbaryl.

mated through Eq. (1) by using the extrapolated k' values of permethrin obtained from plots of log k' vs. % ACN in the mobile phase on the Zorbax C_8 capillary and by considering that the distance traversed by the solvent front of the sample plug is $l_{sp} = 21$ cm. Although the length of the enriched solute band increases from $l_{eb} = 0.0009$ to $l_{eb} = 0.2$ cm as the amount of ACN is increased from 0 to

50% (v/v), the elution step is able to compress the band and to decrease the initial band spreading produced during sample introduction. However, after 50% ACN in the sample plug, the peak height decreased indicating a significant decrease in the effectiveness of the on-line preconcentration. At 50% ACN and higher, not only the initial solute band becomes wider and wider (increasing from $l_{eb}=0.2$ to $l_{eb}=3.8$ cm when going from 50 to 80%, v/v, ACN) but also the elution step is unable to compress the band as the solute molecules move faster and faster in the head sample solvent in which the solute was originally dissolved because its ACN content is increased (see Section 2). In other words, as the amount of ACN in the sample solvent is increased, the difference in the eluent strength between that of the sample solvent and the eluting mobile phase decreases, thus diminishing the focusing effect of the mobile phase during the elution electrochromatography step.

Similar behavior was obtained on the single phase capillary column, see Fig. 6a. As can be seen in Fig. 6a, the maximum is approximately at 50% ACN compared to Fig. 6b where the peak height is at a maximum around 45% ACN. The slightly higher ACN content required for maximum peak height on the single phase ODS capillary than on the segmented Zorbax $C_8 \rightarrow$laboratory-made ODS Nucleosil capillary is due to the longer octadecyl chains which necessitate more organic solvent to open the hydrophobic chains. The C_8 chains on the Zorbax C_8 being shorter than the C_{18} ligands can withstand higher aqueous concentration without undergoing significant stack configuration (see Fig. 5 for illustration). As shown in Fig. 7, 50% ACN in the sample solvent seems to be optimal for trace enrichment of the pyrethroids.

In our previous contribution to trace enrichment in CEC [9], the optimum organic modifier concentration in the sample solution was determined to be 30% for the laboratory-made ODS Nucleosil using methiocarb as the model solute for carbamate insecticides. In the present study, the effect of ACN in the sample plug was studied on a segmented column (Zorbax $C_8 \rightarrow$laboratory-made ODS Nucleosil) using carbaryl as the model solute. The results are shown in Fig. 7c by a plot of peak height vs. % ACN in the injection sample. The sample plug was introduced electrokinetically at 20 kV from a sample concentration of $1.3\cdot10^{-6}$ *M* in carbaryl for an injection time of 3.5 min. When comparing Fig. 7c to b, one can see the same trends. Due to the fact that the carbamates are less hydrophobic than the pyrethroids, the percent organic in the sample injection is less for the carbamates (30% ACN) than for the pyrethroids (45% ACN) for maximum peak height obtained in the subsequent EEC step.

4.2.2. Sensitivity enhancement under optimal conditions

Tables 1 and 2 list the limits of detection (LODs) with and without on-column trace enrichment for carbamates and pyrethroid insecticides, respectively, under various column and detection configurations.

Table 1
Limit of detection with or without preconcentration for two carbamate insecticides

Solute/ wavelength	No preconcentration (*M*), 2 s injection at 20 kV		Preconcentration (*M*), 3.5 min injection at 20 kV		
	On-capillary detection	High sensitivity cell	On-capillary detection	High sensitivity cell	Segmented capillary with high sensitivity cell
Carbaryl					
282 nm	$2.24\cdot10^{-5}$	$8.95\cdot10^{-6}$	$7.95\cdot10^{-8}$	$4.48\cdot10^{-8}$	$2.98\cdot10^{-8}$
208 nm	$6.34\cdot10^{-6}$		$5.63\cdot10^{-8}$		$1.83\cdot10^{-8}$
Methiocarb					
282 nm	$5.54\cdot10^{-6}$	$1.51\cdot10^{-5}$	$8.93\cdot10^{-8}$	$6.04\cdot10^{-8}$	$5.03\cdot10^{-8}$
208 nm	$4.33\cdot10^{-6}$		$3.85\cdot10^{-8}$		$1.25\cdot10^{-8}$

All samples were made in deionized water. Conditions for elution: mobile phase, 10 m*M* ammonium acetate (pH 6.0)–ACN (20:80, v/v); voltage, 20 kV; other conditions as in Fig. 8.

Table 2
Limit of detection with and without preconcentration for some pyrethroids

Solute	No preconcentration (M), 2 s injection at 20 kV		Preconcentration (M), 3.5 min injection at 20 kV		
	On-capillary detection	High sensitivity cell	On-capillary detection	High sensitivity cell	Segmented capillary with high sensitivity cell
Fenpropathrin	$6.20\cdot10^{-6}$	$2.98\cdot10^{-6}$	$4.96\cdot10^{-8}$	$2.17\cdot10^{-8}$	$1.15\cdot10^{-8}$
Sanmarton	$8.73\cdot10^{-6}$	$3.93\cdot10^{-6}$	$6.55\cdot10^{-8}$	$3.49\cdot10^{-8}$	$1.42\cdot10^{-8}$
Permethrin	$5.72\cdot10^{-6}$	$2.06\cdot10^{-6}$	$3.43\cdot10^{-8}$	$1.96\cdot10^{-8}$	$7.00\cdot10^{-9}$

All samples were made in deionized water. Conditions for ''No preconcentration'' as in Fig. 6a and for ''Preconcentration'' as in Fig. 8.

First, without preconcentration and as expected, the LOD decreased in the presence of the z-cell configuration by 2.5- and 3.7-fold for carbaryl and methiocarb, respectively, at 282 nm. Similarly, for fenpropathrin, sanmarton and permethrin, the LOD decreased by 2.1-, 2.2- and 2.7-fold, respectively, at 214 nm. As can be seen in Tables 1 and 2, significant sensitivity enhancement was achieved by on-column trace enrichment. In the case of carbamate insecticides, and under optimum conditions, 282- and 620-fold sensitivity increase were obtained with carbaryl and methiocarb, respectively, with on capillary detection at 282 nm. This sensitivity enhancement grew 500- and 917-fold for carbaryl and methiocarb, respectively, when the capillary was connected to the z-cell. The segmented capillary with a z-cell at 282 nm boosted the sensitivity to 752- and 1100-fold for carbaryl and methiocarb, respectively. At 208 nm, although the sensitivity enhancement was smaller, ca. 112-fold for both carbaryl and methiocarb with on-capillary detection and 346-fold for combined segmented capillary and z-cell, more dilute samples were determined at 208 nm, see Table 1. In the case of pyrethroid insecticides, 125-, 133- and 167-fold increases in sensitivity were obtained by the trace enrichment of fenpropathrin, sanmarton and permethrin, respectively, under optimal conditions using on-capillary detection. This sensitivity enhancement became 286-, 250- and 292-fold for fenpropathrin, sanmarton and permethrin, respectively, when using the z-cell for detection. This sensitivity increase was further enhanced by combining segmented capillary with the z-cell and grew to 539-, 615- and 817-fold for fenpropathrin, sanmarton and permethrin, respectively.

As expected, the magnitude of sensitivity enhancement paralleled the order of solute retention. In other words, the more retained the solute the more effective the trace enrichment.

4.2.3. Simultaneous on-line preconcentration and separation of pyrethroids and carbamates spiked in deionized, tap and lake waters

Fig. 8a–c show typical electrochromatograms for the simultaneous on-line preconcentration and separation of fenpropathrin, sanmarton and permethrin spiked in deionized, tap and lake waters, respectively. In all cases, the water sample was spiked with the three pyrethroids at a concentration of 10^{-8} M for each solute in a solvent composed of acetonitrile–buffer (50:50, v/v). While the deionized water sample was injected for 3.5 min at 20 kV that of tap and lake waters was introduced at the same voltage but for 4.1 and 5.0 min, respectively. The elution and separation were then achieved at 20 kV with a mobile phase of THF–ACN–14 mM ammonium acetate, pH 6.0 (5:80:15, v/v). The injection time in the case of tap water was prolonged to 4.1 min due to the fact that the EOF in the presence of tap water in the blank and sample decreased by a factor of 1.17 with respect to that with deionized water under otherwise the same running conditions. Under these circumstances, prolonging the injection time with tap water to 4.1 min insured the introduction of the same sample plug length as in the case of deionized water (i.e., 21 cm sample plug). Also, the EOF in the presence of lake water was almost identical to that of tap water. The injection for lake water was prolonged for an extra 0.9 min with respect to tap water due to an interfering peak in the electrochromatogram when the sample is introduced for 4.1 min as in the case of tap water. As shown in the blank

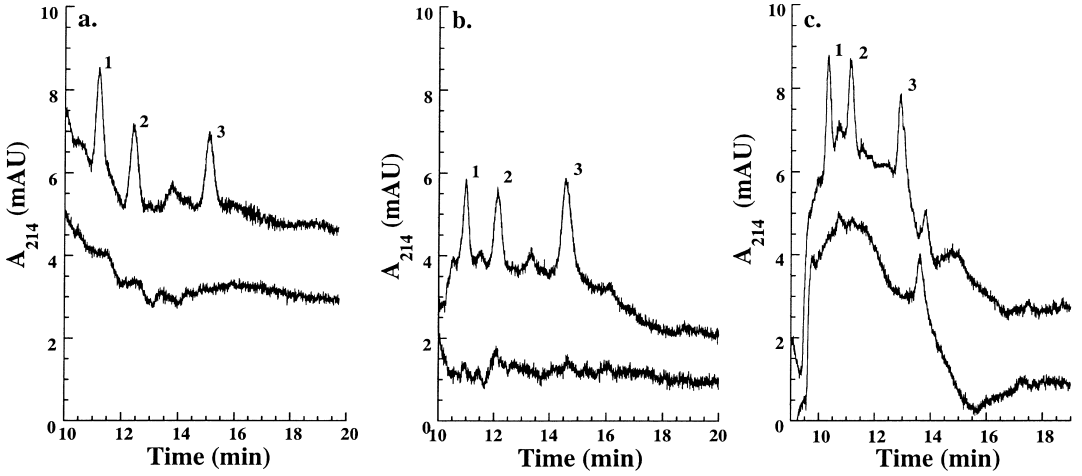

Fig. 8. Simultaneous on-line preconcentration and separation of pyrethroids spiked in deionized (a), tap (b) and lake (c) waters. Conditions of frontal electrochromatography: injection time, 3.5 min in (a) 4.1 min in (b) and 5.0 min in (c); injection voltage, 20 kV; solutes: 1, fenpropathrin; 2, sanmarton; 3, permethrin at ca. 10^{-8} *M* spiked in deionized, tap or lake water at 50% (v/v) ACN. Conditions of elution electrochromatography: voltage, 20 kV; mobile phase, 14 m*M* CH_3COONH_4 (pH 6.0)–acetonitrile–THF (15:80:5, v/v). High sensitivity cell used for detection; 33.5 cm capillary with concentrating segment of 2.5 cm packed with Zorbax C_8 and a separation segment of 22.5 cm packed with laboratory-made ODS Nucleosil.

and sample electrochromatograms in Fig. 8c, prolonging the injection time by an extra 0.9 min moved the interfering peak away from the analyte peak 3. According to our previous findings [9], a longer injection than 4.1 min or 21 cm would not yield a significant increase in peak height. The lower EOF in the case of natural waters (e.g., tap water and lake water) may be attributed to the presence of various inorganic ions, specially the divalent calcium ion, and humic and fulvic substances [16,17] which collectively confer to natural waters a higher ionic strength and higher viscosity than deionized water.

Under optimal conditions using segmented capillary with high sensitivity cell, the trace enrichment by sequential frontal and elution electrochromatography allowed the detection of very dilute samples of pesticides prepared in deionized, tap and lake waters. At 208 nm, the LODs for carbaryl in deionized, tap and lake waters were $1.83\cdot10^{-8}$, $1.97\cdot10^{-8}$ and $2.53\cdot10^{-8}$ *M*, respectively. Under the same trace enrichment conditions, the LODs for methiocarb in deionized, tap and lake waters were $1.2\cdot10^{-8}$, $1.35\cdot10^{-8}$ and $1.73\cdot10^{-8}$ *M*, respectively. These figures which are very close in magnitude demonstrate that the trace enrichment is not influenced by the nature of the water matrix. Also the LODs for the three pyrethroids were largely independent of the nature of the water matrix. In fact, the LODs for fenpropathrin in deionized, tap and lake waters were found to be $1.15\cdot10^{-8}$, $1.14\cdot10^{-8}$ and $1.14\cdot10^{-8}$ *M*, respectively. The LODs for sanmarton were $1.42\cdot10^{-8}$, $9.76\cdot10^{-9}$ and $9.76\cdot10^{-9}$ *M* while the LODs for permethrin were found to be $7.0\cdot10^{-9}$, $1.21\cdot10^{-8}$ and $1.21\cdot10^{-8}$ *M* in deionized, tap and lake water, respectively.

5. Conclusions

Capillary electrochromatography with segmented packed capillary columns using a high sensitivity cell (i.e., z-cell) allows the rapid and efficient separation of closely related pesticides and the introduction of a relatively large sample plug equivalent to almost one column dead volume. This permitted the determination of dilute samples of carbamate and pyrethroid insecticides consisting of tap and lake water spiked at concentration of $\sim10^{-8}$ to 10^{-9} *M* with a UV detector. These LODs were largely independent of the nature of the water matrix.

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