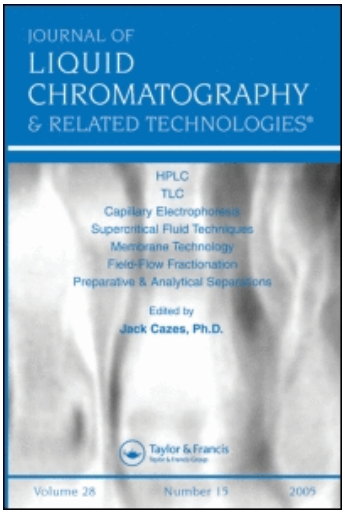

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On-Line Preconcentration of Triazine Herbicides with Tandem Octadecyl Capillaries - Capillary Zone Electrophoresis

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ON-LINE PRECONCENTRATION OF TRIAZINE HERBICIDES WITH TANDEM OCTADECYL CAPILLARIES-CAPILLARY ZONE ELECTROPHORESIS*

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ABSTRACT

Fused-silica capillaries having surface-bound octadecyl functions were developed for on-line preconcentration of dilute samples prior to capillary zone electrophoresis. The performance of tandem octadecyl capillaries-capillary zone electrophoresis was evaluated with solutes of environmental significance, e.g., prometon and prometryne. The on-line preconcentration was best achieved when oligomeric octadecyl capillaries having roughened inner walls were employed. The coupled configuration enhanced the detectability in terms of solute concentration by a factor of 10 to 35 as compared to that obtained by capillary zone electrophoresis alone. Large volumes of samples could be introduced without affecting separation efficiency.

INTRODUCTION

Capillary zone electrophoresis (CZE) employs high electric fields to yield rapid separations. In order to dissipate Joule heating resulting from the passage of current

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through the electrolyte inside the tube, capillaries of 25-100 μm I.D. are used. With such small diameter capillary tubes, the injection volume must be small (*i.e.*, 1-5 nL) in order to achieve high separation efficiencies. For this reason dilute samples cannot be determined by capillary zone electrophoresis with concentration sensitive detectors. They must be concentrated so that the thin plug injected would contain a detectable amount of the analytes.

Although this problem has been recognized since the introduction of capillary zone electrophoresis, only few attempts have been made to render the technique suitable for the determination of dilute samples. Recently, on-line preconcentration by isotachophoresis (1-7) and electrophoresis (8) were introduced. However, these techniques have some drawbacks. The isotachophoretic concentration mode is difficult to automate and is limited by the choice of electrophoresis buffers. Also, positive and negative analytes cannot be determined at the same time. The electrophoretic concentration method has a limited loadability, which means only small sample volume can be introduced.

This report is concerned with the investigation of the potentials of on-line preconcentration with capillaries having interactive walls. In this regard, fused-silica capillaries with surface-bound octadecyl functions for on-line preconcentration of dilute samples of environmental significance were developed. In general, on-line preconcentration has several advantages over off-line preconcentration. With on-line preconcentration, sample contamination and decomposition can be effectively minimized. As it will be demonstrated in this report, with interactive preconcentration open tubular capillaries, a large amount of sample can be introduced without significant loss in separation efficiency, and consequently low detection limit in terms of sample concentration can be achieved.

SOME PRINCIPLES OF ON-LINE PRECONCENTRATION WITH OPEN TUBULAR OCTADECYL CAPILLARIES

On-line preconcentration with interactive capillaries is an adsorption process that can be described by an adsorption isotherm. An adsorption isotherm provides a relationship between the concentration of the solute in the bulk solution and the amount of solute adsorbed onto the sorbent when the two phases are in equilibrium. Two types of adsorption isotherm are typically used in describing liquid-solid adsorption; Langmuir and Freundlich. The Langmuir isotherm is convenient for the quantitative analysis of adsorption processes and has a physico-chemical basis while the Freundlich model is

purely empirical. For the discussion of on-line preconcentration data, the Langmuir model is considered, which can be expressed by the equation :

$$q = \frac{ac}{1 + bc}$$

where q is the amount of solute in the adsorbed phase, c is the concentration of the solute in the bulk liquid phase, and b is an empirical constant related to the energy of adsorption. At low solute concentration, $bc << 1$ and $q \approx ac$. Under these conditions, a linear isotherm is obtained with a slope equal to parameter a, which is the equilibrium constant for the sorption process, K. At high solute concentration, $bc >> 1$ and the Langmuir isotherm flattens out, i.e., q approaches q_{max}. This means that at high solute concentrations the adsorption sites become saturated with adsorbed solute molecules, and the concentration of the solute in the adsorbed phase approaches a maximum value. Since the solute concentrations were relatively low in the on-line preconcentration studies, linear isotherms were expected.

The capillary used in this study consisted of two sections connected with a Teflon tube having an inner diameter of the same size as the outer diameter of the two capillaries. The first part is a preconcentration capillary, *i.e.*, an open-tubular reversed-phase chromatography column with bonded octadecyl functions on the inner wall. The second part is a separation capillary, *i.e.*, a CZE capillary.

As the sample is introduced, it first enters the preconcentration capillary, and is accumulated on the interactive walls. Samples are retained by the octadecyl groups by hydrophobic interaction. Since this type of interaction is non-selective, octadecyl capillaries can be applied for a wide variety of compounds provided that they have non-polar functions.

The on-line preconcentration process involves two consecutive steps: the accumulation of the solute onto the walls of the preconcentration capillary, and stripping the accumulated solute off the capillary walls. The accumulation of solutes on the octadecylsilyl walls from dilute samples should be carried out in the presence of an electrolyte (e.g. aqueous solution) that affords the strongest interactions between the analyte and the interactive walls, i.e., a binding electrolyte. In the desorption step, the accumulated solutes on the walls of the preconcentration capillary should be stripped off the walls with a strong debinding electrolyte (e.g. hydro-organic solution) so that they enter the separation capillary as a thin plug whereby separation starts. The binding electrolytes used in this study were sodium phosphate solutions, whereas the debinding electrolytes were sodium phosphate solutions containing acetonitrile. Acetonitrile served as the debinding agent.

EXPERIMENTAL

Instruments and Capillary Tubes

The capillary electrophoresis instrument used in this study closely resembles that described earlier (9-11). It consists of a 30-kV dc power supply Model EH30P03 of positive polarity from Glassman High Voltage (Whitehouse Station, NJ, U.S.A.) and a Linear (Reno, NV, U.S.A.) Model 200 UV-Vis variable wavelength detector equipped with a cell for on-column detection. The electropherograms were recorded with a computing integrator from Shimadzu (Columbia, MD, U.S.A.).

The instrument for the UV spectrometry measurement was a UV-Visible Recording Spectrophotometer, Model UV-160 from Shimadzu.

Fused-silica capillaries having an inner diameter of 50 μm and outer diameter of 375 μm were obtained from Polymicro Technology (Phoenix, AZ, U.S.A.). The capillary used in this study consisted of two short capillaries connected in series. The first one was the preconcentration capillary, which had a length of 20 cm and an inner diameter of 50 μm. The second one was the separation capillary, which had a total length of 60 cm with 30 cm to the detection point. Untreated fused-silica capillaries were used as the separation capillary.

Reagents and Materials

The herbicides, prometon and prometryne were purchased from Chem Service (West Chester, PA, U.S.A.). Ammonium hydrogen bifluoride, sodium phosphate dibasic, phosphoric acid, hydrochloric acid, toluene, acetone, methanol and acetonitrile (both HPLC grade) were from Fisher Scientific Company (Fair Lawn, NJ, U.S.A.). Dimethyloctadecylchlorosilane and octadecyltrichlorosilane were from Petrarch Systems Inc (Bristol, PA, U.S.A.). 2-Naphthol was purchased from Aldrich (Milwaukee, WI, U.S.A.). Colloidal silica, Ludox HS-40, was a gift from Du Pont (Willmington, DE, U.S.A.).

Capillary Surface Modification

The inner surface of the preconcentration capillaries was roughened before the bonding of the interactive functions. The purpose of this treatment is to increase the specific surface area of the capillary wall and in turn the concentration of the interactive functions on the surface, so that a larger amount of the analytes can be accumulated. This treatment involved etching, and in most cases, subsequent coating with colloidal silica.

In the etching process, the capillaries were filled with a 5% (w/v) solution of ammonium hydrogen bifluoride in methanol and allowed to stand for 1 hr before the

solution was removed with a flow of nitrogen gas (12). The capillaries were then sealed in flame and heated at 250 °C or 300 °C for 5 hrs. At high temperature, ammonium hydrogen bifluoride dissociates to produce gaseous hydrogen fluoride and ammonia (12). Thereafter, the capillaries were flushed with 0.01 M HCl, water and finally stored in HPLC grade methanol. Etching of fused-silica with hydrogen fluoride has been shown to produce pits of different diameters on the surface (13).

To prepare support coated capillaries, the etched tubes were filled with a 10% (w/v) colloidal silica solution and heated at 250 °C for 1 hr. This treatment was repeated 3 times and finally the capillaries were stored in HPLC grade methanol.

The preparation of capillaries with surface-bound octadecyl functions was carried out as follows: the etched and/or support coated capillaries were filled with a solution of 0.2 g/mL dimethyloctadecylchlorosilane or octadecyltrichlorosilane in toluene, and heated at 110 °C for 1 hr. This treatment was repeated twice. After this treatment, the capillaries were flushed with acetone and stored in HPLC grade methanol.

The octadecyl capillary tubes thus obtained are denoted by monomeric-ODS-Cap., or oligomeric-ODS-Cap., to distinguish between capillaries coated with dimethyloctadecylchlorosilane or octadecyltrichlorosilane, respectively.

UV Spectrophotometry Measurements

Sample solutions were made by dissolving a small amount of prometon and prometryne in deionized water containing 10% (v/v) acetonitrile to enhance the solubility of the herbicides. The absorption spectra of prometon and prometryne were obtained by scanning from 200 to 350 nm. The wavelengths of maximum absorbance, λ_{max}, for prometon and prometryne were found at 220 and 223 nm, respectively. For this reason, the UV detector for capillary electrophoresis was set at 220 nm.

Chromatographic Measurements with Open-Tubular Octadecyl Columns

To determine the retentivity of the octadecyl preconcentration capillaries toward the pollutants under investigation, a gravity-driven flow was used for both sample injection and elution of the analyte. The reservoir at one end was raised to 20 cm above the outlet reservoir. Sodium nitrate was used as the inert tracer, since it is not retained by the octadecyl preconcentration capillary and detects well in the UV. The retention factor was calculated using the following equation:

$$k' = \frac{t_R - t_0}{t_0}$$

where t_R and t_0 are the retention times of the solute and the the inert tracer, respectively.

Binding and Debinding Processes

The binding electrolytes were 50 mM phosphate solutions whereas the debinding electrolytes were 50 mM phosphate solutions at various concentrations of acetonitrile. Dilute samples of the herbicides and 2-naphthol were prepared in the binding electrolyte. All samples and electrolytes were filtered with 0.2 μm Uniprep Syringeless filters from Genex Corp., (Gaithersburg, MD, U.S.A.) to avoid capillary plugging.

In on-line preconcentration experiments, sample introduction (or feeding) was carried out by either electromigration or hydrodynamic flow at the anode end. When the electromigration mode was used for sample introduction, the injection voltage was the same as that for the separation, and the debinding electrolyte was also introduced by electromigration. When the hydrodynamic mode was used, sample reservoir was raised to a certain height above the outlet reservoir. The debinding electrolyte was allowed to flow by gravity for the same period of time as that for the sample introduction. Thereafter, the reservoir at the anode end was lowered to the same height as that of the outlet reservoir and the voltage was applied.

Between runs, the capillaries were flushed successively with acetonitrile, water, and the binding electrolyte. The capillaries were allowed to equilibrate for 10 to 20 minutes with the binding electrolyte before each run.

RESULTS AND DISCUSSION

Detection Limit with CZE

To evaluate the effectiveness of the preconcentration approach under investigation in the determination of dilute samples, it was first necessary to examine the potential of CZE alone, i.e., without an on-line preconcentration capillary. Under normal injection conditions, *i.e.*, the sample was introduced as a thin plug, the detection limits for both prometon and prometryne were 1 μg/mL, *i.e.*, 4.4 and 4.1 x 10^{-6} M for prometon and prometryne, respectively. This set of experiments shows that a means for preconcentration is necessary in order to further decrease the concentration detection limit.

Open-Tubular Chromatography with Preconcentration Capillaries

The retentivity of octadecyl capillaries toward the solutes of interest was determined by elution chromatography with a gravity-driven flow (see experimental for details). Figure 1 illustrates the results obtained with prometon, prometryne and 2-naphthol by plots of retention factor, k', versus percent acetonitrile (v/v) in the eluent. As shown in Fig. 1, prometon and prometryne were more retained than 2-naphthol may be due

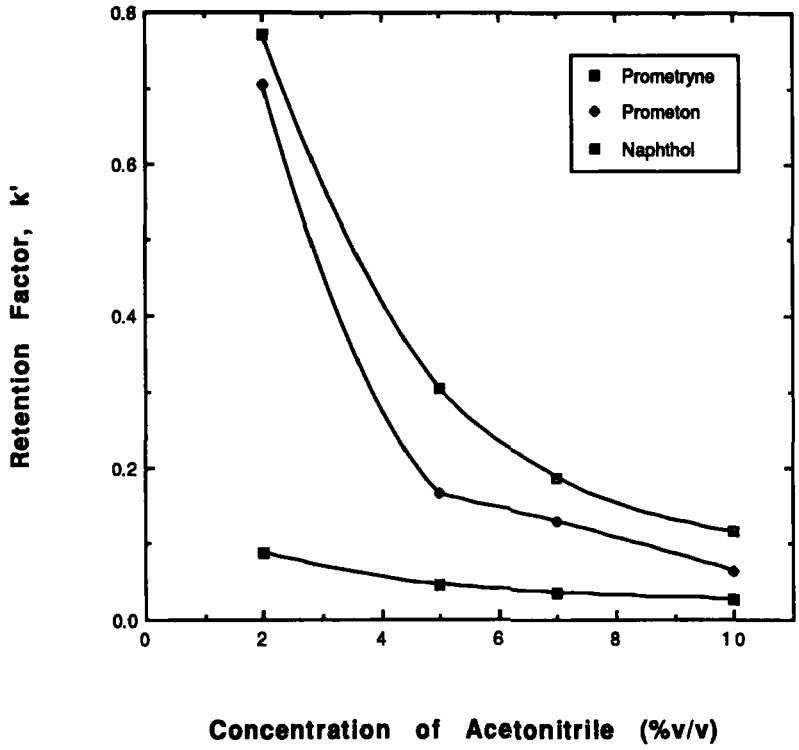
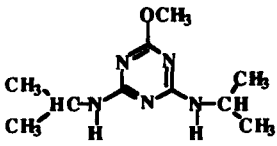
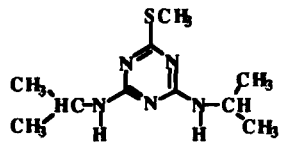


FIGURE 1. Retention factor, k', as a function of acetonitrile concentration in the mobile phase. Preconcentration capillary, oligomeric-ODS-Cap., etched at 300 °C, 20 cm x 50 μm I.D.; separation capillary, untreated, 30 cm (to the detection point), 60 cm (total length) x 50 μm I.D.; eluent, 10 mM sodium phosphate, pH 6.0, at various percent acetonitrile (v/v); sample injection, hydrodynamic, Δh = 20 cm, 10 seconds; inert tracer, sodium nitrate; detection, 220 nm.



Prometon



Prometryne

to the presence of the two isopropyl groups in the herbicides, which imparted them with stronger hydrophobic character. The only difference in the structures of the two herbicides

is that the methoxy group in prometon is replaced by a methylthio group in prometryne. Since oxygen is more electronegative than sulfur, the methoxy group is more polar than methylthio group. Therefore, prometryne is more hydrophobic than prometon. This may explain the slightly higher retention of prometryne. In all cases, the retention factors decreased rapidly with increasing acetonitrile concentration in the mobile phase, see Fig. 1.

The above experiments show the ability of octadecyl preconcentration capillaries to retain the solutes under investigation when the mobile phase is a neat aqueous buffer. It also shows that relatively high concentration of acetonitrile is needed to elute accumulated solutes from the capillary wall.

Effect of Operational Parameters

In order to determine the optimum conditions for the preconcentration with octadecyl capillaries, 2-naphthol was chosen as a model solute to study the effects of various operational parameters. These parameters are the capillary surface treatment, concentration of debinding agent, and feeding time.

Capillary treatment. The inner surface area of the preconcentration capillaries was increased by chemical and/or physical treatments prior to the covalent attachment of the non-polar stationary phase (see experimental for details). In addition, monomeric or oligomeric octadecyl capillaries were prepared by reacting the roughened surface either with dimethyloctadecylchlorosilane or with octadecyltrichlorosilane, respectively. The effects of the surface pretreatments as well as the nature of the non-polar coatings, i.e., monomeric or oligomeric octadecyl coatings, on the effectiveness of the various preconcentration capillaries were investigated. The results for 2-naphthol are illustrated in Fig. 2 in terms of peak height versus sample concentration. For comparison, the results obtained with CZE alone *i.e.*, without an on-line octadecyl preconcentration capillary, are also shown. As expected, for the same sample concentration and feeding time, the etched capillary having oligomeric octadecyl coating yielded higher signal than the one with monomeric octadecyl coating; compare curves 2 and 3 in Fig. 2. Trifunctional silanizing reagents can form oligomeric coatings on the surface of the fused-silica capillary , thus providing more sites per unit surface area for the binding of the analyte. Further improvement in the analytical signal was obtained when the capillary was etched and support coated prior to the bonding of octadecyltrichlorosilane. The deposition of colloidal silica on the etched surface would yield a porous layer of large surface area, and as a result, the concentration of octadecyl functions per capillary tube would increase.

The above experiments were performed in order to determine the optimum surface modification that would yield the highest signal from a given sample concentration in the feeding solution rather than to determine the concentration detection limit. As can be seen

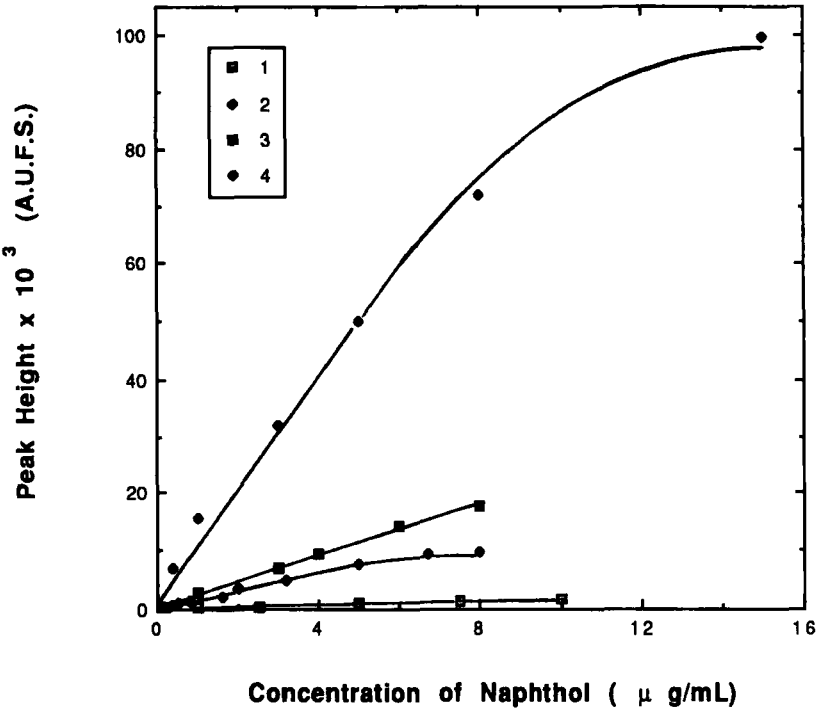


FIGURE 2. Effects of capillary surface treatments. Separation capillary as in Fig. 1. Curve 1, CZE without on-line preconcentration, injection by electromigration, 10 seconds; curves 2, 3 and 4, on-line preconcentration with various octadecyl capillaries, 20 cm x 50 μm I.D.. Curve 2, monomeric-ODS-Cap. etched at 250°C; curve 3, oligomeric-ODS-Cap. etched at 250 °C; curve 4, oligomeric-ODS-Cap. etched at 250 °C, support coated. Binding electrolyte, 50 mM phosphate, pH 6.5; debinding electrolyte, 50% (v/v) acetonitrile in binding electrolyte; sample, 2-naphthol; running voltage, 15 kV; detection, 226 nm; sample introduction, electromigration; feeding time, 5 min.

in Fig. 2, the signal obtained with CZE alone was much less than that obtained with on-line preconcentration. With octadecyl preconcentration capillaries, the detectability of CZE can be increased by a factor of 15-35, when the surface coverage with octadecyl functions is high; see curve 4 in Fig. 2. In all cases involving preconcentration, the volume of sample introduced was ca. 100-120 nL.

It has to be noted that the preparation of octadecyl capillaries with deposited colloidal silica on the inner walls was rather complicated by the frequent plugging of some portions of the tube, which on the average made it difficult to generate more than one open

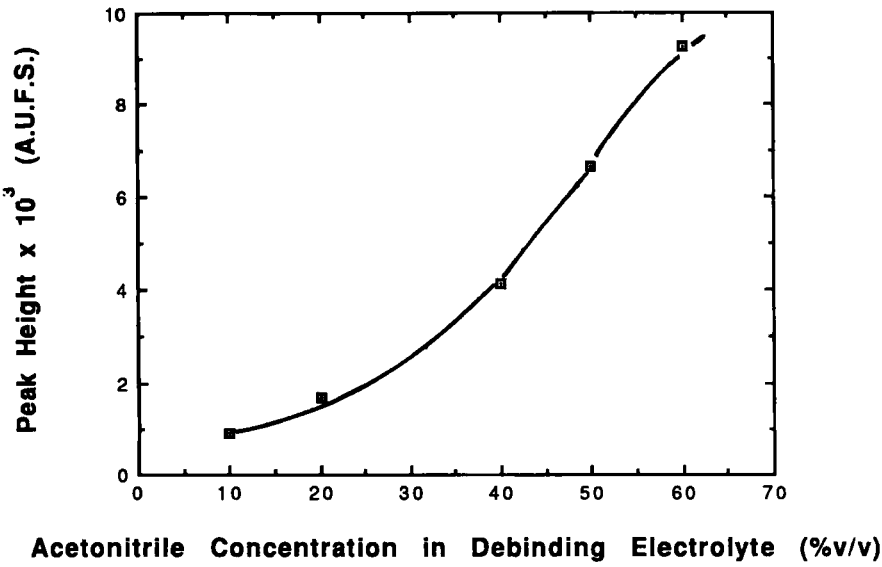


FIGURE 3. Effect of acetonitrile concentration in the debinding electrolyte. Preconcentration capillary, monomeric-ODS-Cap, etched at 250 °C , 20 cm x 50 μm I.D.; separation capillary, same as in Fig. 1; binding electrolyte, 50 mM sodium phosphate, pH 6.5; debinding electrolyte, acetonitrile in binding electrolyte; sample, 2-naphthol, 1.3 μg/mL; sample introduction, electromigration; feeding time, 5 min; running voltage, 15 kV; detection, 226 nm.

tube for every five capillaries. Another alternative for preparing capillaries with high inner surface areas was to use a higher temperature during the etching step. In fact oligomeric octadecyl-capillaries with etched surface at 300 °C instead of 250 °C yielded comparable performance to those with surface etched at 250 °C and support coated. The oligomeric octadecyl-capillaries with surface etched at 300 °C were then used for the preconcentration of herbicides (see later).

With on-line octadecyl preconcentration capillaries (see curves 2, 3 and 4 in Fig. 2), peak height increased linearly with sample concentration for up to 5-8 μg/mL. This illustrates the usefulness of tandem octadecyl capillaries --> CZE in the quantitative determination of dilute samples. The linear relationship between peak height and feed concentration at relatively low solute concentration is an indication of a linear adsorption isotherm and is in agreement with the Langmuir model for liquid-solid adsorption (see above).

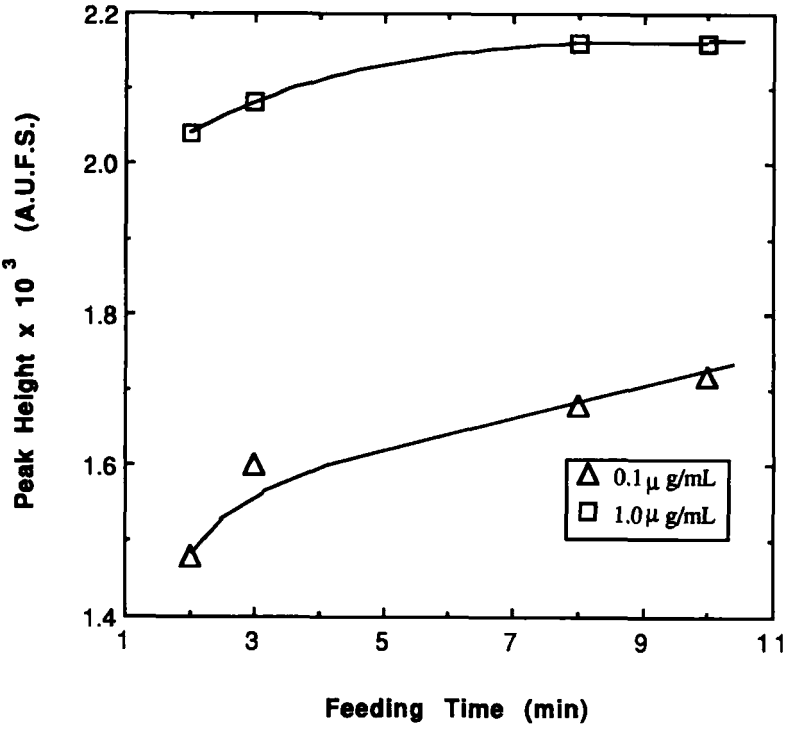


FIGURE 4. Effect of feeding time. Debinding electrolyte, 50% (v/v) acetonitrile in binding electrolyte; sample, 2-naphthol; other experimental conditions are as in Fig. 3.

On the other hand, curve 4 in figure 2, which was obtained over a wider range of sample concentration, reflects an adsorption isotherm of the Langmuir type and shows that at concentration higher than 10 μg/mL the linear dependency between peak height and feed concentration is no longer conserved indicating a beginning of saturation of the adsorption sites with the solute molecules.

Concentration of debinding agent. In this study, acetonitrile was chosen as the debinding agent. Figure 3 shows the effect of acetonitrile concentration in the debinding electrolyte on the peak height of desorbed 2-naphthol. As expected, the signal increased with the concentration of acetonitrile. This means that a high concentration of debinding agent can result in a better recovery of the sample. However, a high concentration of organic solvent led to a large breakthrough signal, which caused problem in the determination of very dilute samples.

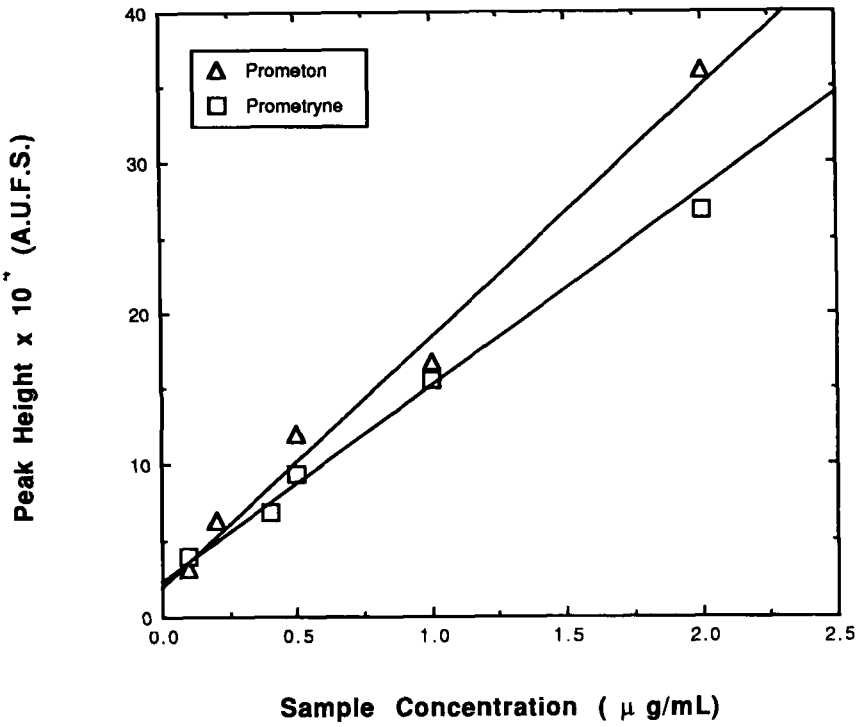


FIGURE 5. Quantitative determination of dilute sample of prometryne and prometon with octadecyl preconcentration capillaries. Preconcentration capillary, oligomeric-ODS-Cap, etched at 300 °C, 20 cm x 50 μm I.D.; separation capillary, same as in Fig. 1; binding electrolyte, 10 mM sodium phosphate, pH 6.0; debinding electrolyte, 50% (v/v) acetonitrile in binding electrolyte; sample introduction, hydrodynamic; feeding time, 5 min; running voltage, 15 kV; detection, 220 nm.

Feeding Time. The effect of feeding time is shown in Fig. 4. As the feeding time increased the signal increased rapidly first and then leveled-off. This means that an adsorption equilibrium was reached between the feed and the inner surface of the preconcentration capillary, and further increase in the feeding time did not lead to more accumulation of the solute. As expected, the optimum feeding time decreased as the concentration of the feed solution increased. This can be explained by the fact that adsorption from bulk solution is a diffusion controlled process.

Quantitative Determination of Dilute Samples of Herbicides with Octadecyl Preconcentration Capillaries

To examine the usefulness of tandem octadecyl capillary-->CZE in the quantitative determination of environmental pollutants from dilute samples, and to assess the detection limit in terms of concentrations using the tandem configuration, prometon and prometryne were employed as the model herbicides. The results are depicted in Fig. 5 by plots of peak height versus sample concentration. As can be seen in Fig. 5, peak height increased linearly with sample concentration in the range studied. The detection limit for both prometon and prometryne were approximately 0.1 μg/mL (*i.e.*, 4.4 and 4.1 x 10^{-7} M for prometon and prometryne, respectively), which is 10 folds lower than that of CZE alone, under otherwise the same experimental conditions.

CONCLUSION

On-line preconcentration with octadecyl-capillaries enhanced the analytical signal by a factor of 10-35 with concentration sensitive detectors as compared to that obtained with CZE alone. The octadecyl preconcentration capillaries require small amounts of the sample and permit continuous sample loading, *i.e.*, large sample volume can be introduced and consequently low detection limit in terms of concentration can be obtained. Furthermore, with preconcentration capillaries plots of peak height versus sample concentration are linear over a wide range, which allow the quantitative determination of dilute samples. Moreover, the on-line preconcentration with interactive capillaries involves small amounts of interactants, and requires simple instrumentation that are customarily used in CZE. These features make the on-line preconcentration technique developed in this work potentially useful in the area of analytical chemistry.

ACKNOWLEDGMENTS

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