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Rapid Screening of Complex Chemical Samples via Capillary Array Analysis

D. S. Anex and D. W. Neyer

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Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

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D. S. Anex and D. W. Neyer
Chemical Sensor Sciences Department
Sandia National Laboratories
P. O. Box 969
Livermore, CA 94551-0969

Abstract

This report is a summary of the results of a two-year Laboratory-Directed Research and Development (LDRD) project that developed instrumentation and methods for capillary array analysis. During the course of this project, a new capillary array electrochromatography instrument was developed to perform eight simultaneous separations and provide complementary chromatographic information from each column on a single sample. The instrument generates eight simultaneous electrochromatograms using a computer-controlled scanning mirror to step the focused output of a 257 nm excitation laser among the capillaries in rapid succession. It uses a parabolic reflector to collect the fluorescence and direct it to a photomultiplier tube. Using this instrument, different samples may be run on each column simply to increase sample throughput. More sophisticated experiments involve running the same sample simultaneously on all columns with different chromatographic conditions. Varying the mobile phase among columns can be used to mimic gradient elution or to speed method development. In addition, use of different stationary phases can be used to perform several simultaneous analyses on one sample. These types of separations have been demonstrated using test mixtures of polycyclic aromatic hydrocarbons, their nitrogen-containing analogues and extracts of creosote-contaminated soil.

Acknowledgment

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Nomenclature

ACN	Acetonitrile
CEC	Capillary Electrochromatography
DI	De-ionized
DNA	Deoxyribonucleic Acid
HPLC	High Performance Liquid Chromatography
LIF	Laser-Induced Fluorescence
NCAC	Nitrogen-Containing Aromatic Compound
NSO	Nitrogen, Sulfur, Oxygen
ODS	Octadecylsilica
PAH	Polycyclic Aromatic Hydrocarbon
PMT	Photomultiplier Tube
RSD	Relative Standard Deviation
USEPA	U.S. Environmental Protection Agency

Rapid Screening of Complex Chemical Samples via Capillary Array Analysis

Introduction

This report describes the development and demonstration of a new capillary array electrochromatography instrument using ultraviolet laser-induced fluorescence (LIF) detection. This instrument simultaneously runs eight capillary electrochromatography (CEC) columns, generating eight electrochromatograms with each instrumental run. Instrument performance was demonstrated using polycyclic aromatic compounds and their nitrogen-containing analogues.

The work described in this report is part of an on-going effort in the development of new analytical techniques based on CEC, [1,2], which has involved gradient elution, [3] fast CEC [4] and the analysis of explosives. [5]

Background

Complete characterization of complex chemical samples often requires multiple analyses. In many cases this involves a number of chromatographic separations involving different stationary phases and various mobile phase compositions. This variation in chromatographic conditions is required to resolve coeluting peaks, which result when there are chemical similarities between molecules. Typically, chromatographic conditions are varied to exploit different chemical selectivities to achieve completely resolved separations of the components of interest. This process is often performed in a serial fashion. In high-performance liquid chromatography (HPLC) a more efficient parallel approach is not practical because of the expense of HPLC pumps and detectors and the complexity of the required liquid connections.

We present a new experimental method for doing multiple, simultaneous, parallel analyses of complex samples using CEC coupled to LIF detection. CEC (and, in general, any of a number of capillary electrokinetic techniques) is naturally suited for simultaneous parallel separations in capillary arrays. CEC has straight forward voltage-driven operation, unsurpassed separation resolution and easy coupling to highly sensitive laser-based optical detection.

Previous instruments based on arrays of capillaries have been generally used to increase sample throughput by running multiple samples through identical columns in an array. For example, our laboratory in collaboration with Beckman Instruments, Inc. (now Beckman-Coulter), developed an eight capillary instrument to perform simultaneous DNA sequencing separations using capillary gel electrophoresis and a two-laser, four-

color LIF detection scheme. This prototype instrument has recently evolved into a commercial product, the Beckman-Coulter CEQ 2000.®

The instrument described in this report has a different emphasis. Rather than running *identical* separations on a number of *different* samples to increase sample throughput (as is the case with the DNA sequencing), this instrument runs *different* separations on a *single* sample. The different separations are designed to maximize the chromatographic information obtained from the sample in a single instrumental run. This can be achieved by varying either the mobile phase composition or the stationary phase among the capillary columns in the array. This parallel chromatographic measurement allows us to identify and characterize an analyte by measurement of its molecular interactions with a wide range of molecular probes (*i.e.*, stationary/mobile phase combinations).

CEC is a high-performance chromatographic technique and is a natural choice for implementation in a parallel chromatographic approach. Since its early demonstration by Pretorius *et al.* [6] and Jorgenson and Lukacs, [7] CEC has gained considerable attention from analytical chemists due to its high efficiency, high selectivity and small sample requirements. CEC employs fused-silica capillary columns packed with an HPLC stationary phase. An axial electric field is applied to the column to propel the mobile phase through the packing. In CEC, the separation mechanism is based primarily on different partitioning of the analytes between the stationary phase and the electroosmotically driven mobile phase. Since it is an electric-field-driven technique, charged species can also separate based on their different electrophoretic mobilities. In CEC, the absence of high column back pressure, which is the case in micro-HPLC, allows the use of longer columns packed with smaller particles ($< 1\mu\text{m}$), leading to dramatic improvements in peak efficiency and sample resolution. Additionally, the plug-like electroosmotic flow profile and the insensitivity of the flow velocity to the dimensions of the interstitial spaces between the particles allows further improvements in separation efficiency.

CEC is well-suited for adaptation into an array format. Since the mobile phase is driven by application of a high voltage, multiple columns can be driven easily from a single power supply. By focusing laser light into the capillaries after the packed portion, very sensitive detection can be achieved using LIF. Detection using LIF is also a good choice for multiple columns. The laser light can be easily steered among the columns by using movable mirrors. The small size of the capillaries lends itself to bundling into an array and facilitates multiple column detection.

As a demonstration of the power of the new capillary array CEC instrument, samples containing 16 polycyclic aromatic hydrocarbons (PAHs) classified as priority pollutants by the U.S. Environmental Protection Agency (USEPA) and 7 nitrogen-containing aromatic compounds (NCACs) were analyzed. Column-to-column and run-to-

run reproducibility were tested using the PAH and NCAC samples and were found to be better than 5% and 1% respectively. Extracts of creosote-contaminated soil were analyzed for the presence of the target PAHs and NCACs.

Experimental

Instrumentation. A schematic of the CEC capillary array instrument is shown in Figure 1. An array of eight fused silica capillary columns (75- μm inner diameter, 365- μm outer diameter, 50-cm total length) packed with 3- μm porous octadecylsilica (ODS) particles (20-cm packed length) are mounted as an array and pass through a parabolic reflector. The capillaries are positioned so that the detection windows are placed near the focus of the reflector. The parabola collects the fluorescence emission and directs it toward the photomultiplier tube (PMT). The inlets and outlets of the capillaries are placed into individual buffer vials. A 0-30-kV high voltage power supply (Bertan High Voltage Corp., Hicksville, NY) provides the electric field for the CEC columns. In the current instrument all inlet electrodes are connected to a common high voltage cable. The capillary outlet reservoirs are in contact with separate ground electrodes, which allows the monitoring of the current in each individual column.

Figure 1: Schematic of the Capillary Array Instrument

An intracavity-frequency-doubled argon ion laser (Coherent, Inc., Santa Clara, CA) operating at 257 nm is used for LIF excitation. A galvometric scanning mirror (General Scanning, Watertown, MA) steps the laser beam from capillary to capillary. A 25-mm focal length fused-silica lens (Melles Griot, Irvine, CA) focuses the laser beam on to the capillary array. The fluorescence signal from the parabolic reflector is passed through a 280-nm long pass filter and is collected by a photomultiplier tube (PMT). A picoammeter (Keithley Instruments, Inc., Cleveland OH) is used to measure the output of the PMT.

The whole experimental process is controlled by a custom-written Labview® (National Instruments Corp., Austin, TX) program running on a notebook computer. Before the run, the laser beam is manually directed to the center of each capillary by adjusting the angle of galvometric scanning mirror using the computer. The control voltage corresponding to the center of each capillary is stored by the computer and is used during data collection to control the stepping of the mirror from capillary to capillary. Typically, the laser is directed at each capillary for 100 ms and quickly stepped between them. The computer collects data points for each capillary in turn and stores the data points in the appropriate electrochromatogram to generate eight simultaneous plots. This results in a point taken every 0.8 sec for each electrochromatogram.

Preparation of the Packed Capillary Array. The columns used in the array are fused silica capillaries (75- μ m inner diameter, 365- μ m outer diameter that are electrokinetically packed with 3- μ m porous ODS. The details of the column packing process have been described previously. [1,3,8]

Materials and chemicals. The fused-silica capillaries were purchased from Polymicro Technologies, Inc. (Phoenix, AZ). The 3- μ m porous ODS packing particles were purchased from SynChrom, Inc. (Lafayette, IN). The priority pollutant PAH mixture (standard reference material, SRM 1647d) was obtained from the National Institute of Standards and Technology. The nitrogen-containing aromatic compounds (NCACs) and the creosote soil extract samples were supplied by William Brumley (USEPA). Acetonitrile (ACN, HPLC grade) was supplied by Aldrich Chemical Co. (Milwaukee, WI). Tris(hydroxymethyl)-aminomethane (TRIS) was supplied by Sigma Co. St. Louis, MO). All DI water used was purified with a Ultra-Pure water system (Labconco Corp., Kansas City, MO). The mobile phase was prepared by mixing 5 mM TRIS, pH 9.0 with appropriate percentage (v/v) of ACN and then fully degassed by vacuum and ultrasonication before use. All samples were made by diluting the standard samples or stock solutions to the desired concentration with the appropriate mobile phase.

Results and Discussion

Gradient-like CEC analysis of complex samples. As a demonstration of the capabilities of the capillary array instrument, a mixture of the 16 USEPA priority pollutant PAHs and the NCACs was analyzed using the multiple capillary instrument. For this demonstration, the same stationary phase (3 μ m ODS) was used in each column and the mobile phase composition was varied between the columns. We term this mode of operation “gradient-like” since the information obtained from the electropherograms mimics that obtained from gradient elution (variation of the mobile phase composition during a single chromatographic run) in a single column. Figure 2 shows a typical electrochromatogram obtained for the PAH/NCAC sample run in gradient-like mode. This analysis varies the percent organic in the mobile phase between columns, using 50%, 60%, 70%, 80% and 90% ACN (balance of the mobile phase is 5 mM TRIS aqueous buffer). As expected, the 90% ACN buffer resulted in fast elution of the sample, but did not resolve the early eluting peaks. With decreasing percentage of ACN, the runs show progressively increasing resolution of the early peaks at the expense of longer retention times for the later peaks. The 50% ACN mobile phase resolves all of the early eluting peaks (including the two components, acenaphthene and fluorene, which usually coelute under conditions of higher percentage organic in the mobile phase), although the later

Figure 2: Gradient-like Separation of a PAH and NCAC Sample

eluting peaks have excessively long retention times. As a result, different columns provide the best information for different ranges of retention times. The complete

chromatographic picture for the sample can be obtained by using information from all the columns, in this case using the information contained in the first ten minutes from each column.

Another interesting observed phenomenon is different peak elution order from columns with different percentage of ACN. For example, in 50% ACN, acridine, 7, 8-benzoquinoline and naphthalene all co-eluted and gave a single peak, while 9-ethylcarbazole, 9-cyanophenanthrene, acenaphthene and fluorene were well resolved. However, with increasing ACN, naphthalene started to come out before the other two co-eluting species. The separation of the three was optimized at 70% ACN with naphthalene eluting first, 7,8-benzoquinoline in the middle and acridine last. The three started to merge again in higher ACN concentration in the mobile phase. This demonstrates the diversity of chromatographic information that can be obtained from a parallel capillary array approach.

Creosote is a significant source of organic contaminants, in particular the PAHs and the NSO compounds, *i.e.* polycyclic aromatic compounds with nitrogen, sulfur or oxygen in the aromatic ring. Creosote-contaminated soil can arise from wood-preservation operations and can pose a significant environmental threat. In order to demonstrate the capability of capillary array CEC for analyzing real life complex mixtures, an extract of creosote-contaminated soil was studied, with the goal of identifying the targeted PAHs and NCACs. Figure 3 shows the separation for the mixture of unknown species in such a sample. In this case, three isocratic mobile phases with different percentages of ACN (60%, 70% and 80%) were used. Some PAH peaks were clearly identified by either spiking them with known sample or with comparisons to the retention times of individual peaks of PAHs and NCACs standard samples. No clear evidence showed there were NCACs in the extract. However, the electrochromatograms clearly prove the high separation power of the gradient-like capillary array CEC approach for the analysis of very complex samples.

Column-to-column and run-to-run reproducibilities. Column reproducibility is always an important issue in CEC analysis. This is especially relevant when we intend to take advantage of the capillary array instrument by running standards and real samples in order to identify unknown components based on their retention times relative to the standards. The run to run reproducibility of the retention times for the mixture of the PAHs and NCACs is shown in Figure 4. The electrochromatograms are from six consecutive runs on one of the eight columns within one day without reloading fresh buffer into the inlet and outlet buffer vials. The relative standard deviations (RSD) in term of retention time were less than 1%. Similar results were obtained from the other 7 columns. As for the column to column reproducibility, Figure 5 shows the electrochromatograms for all eight columns. The RSDs for the retention times in this case were less than 5%. In both cases, benzo[k]fluoranthene was chosen as a model analyte although all other peaks showed similar performance.

Figure 3: Gradient-like separation of an Extract of Creosote-Contaminated Soil

Due to the positions of each column in the array relative to the focal point of the parabolic reflector and PMT, we didn't expect to achieve the similar fluorescence signal intensity from all eight channels. Normally, the emission light intensity from columns near the focal point was 2-3 times higher than those away from the focal point. But the variation for signal to noise ratios from the 8 channels was still within a factor of 2. In the figures, the signal intensity has been normalized.

Figure 4: Run-to-Run reproducibility of the Capillary Array Instrument

Figure 5: Column-to-Column reproducibility of the Capillary Array Instrument

CONCLUSION

In this project, we have demonstrated the effectiveness of the capillary array CEC approach for the analysis of complex samples. The approach not only inherits high-selectivity, high-efficiency and high-universality from single-column CEC, but also offers extra capabilities for very complex samples. Gradient-like methods significantly reduce the time needed for separations of sample with coeluting peaks or components with long retention times and avoid the complexity of single-column gradient elution. Running unknown and internal standard samples simultaneously is very helpful for peak identification and confirmation based on relative retention times. The capillary array approach has the potential to perform multiple-sample analyses to achieve high sample throughput or to greatly increase the chromatographic information obtained by a single sample by varying the stationary phase as well as the mobile phase composition.

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